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Review

# Atomic Force Microscopy and pharmacology: From microbiology to cancerology

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ABSTRACT

*Background:* Atomic Force Microscopy (AFM) has been extensively used to study biological samples. Researchers take advantage of its ability to image living samples to increase our fundamental knowledge (biophysical properties/biochemical behavior) on living cell surface properties, at the nano-scale.
*Scope of review:* AFM, in the imaging modes, can probe cells morphological modifications induced by drugs. In the force spectroscopy mode, it is possible to follow the nanomechanical properties of a cell and to probe the mechanical modifications induced by drugs. AFM can be used to map single molecule distribution at the cell surface. We will focus on a collection of results aiming at evaluating the nano-scale effects of drugs, by AFM. Studies on yeast, bacteria and mammal cells will illustrate our discussion. Especially, we will show how AFM can help in getting a better understanding of drug mechanism of action.
*Major conclusions:* This review demonstrates that AFM is a versatile tool, useful in pharmacology. In microbiology, it has been used to study the drugs fighting *Candida albicans* or *Pseudomonas aeruginosa*. The major conclusions are a better understanding of the microbes' cell wall and of the drugs mechanism of action. In cancerology, AFM has been used to explore the effects of cytotoxic drugs or as an innovative diagnostic technology. AFM has provided original results on cultured cells, cells extracted from patient and directly on patient biopsies.
*General significance:* This review enhances the interest of AFM technologies for pharmacology. The applications reviewed range from microbiology to cancerology.



## 1. Introduction

Historically, imaging at high resolution is based on the optical microscope. However this technique suffers from the limitation of the photons wavelength, roughly 200 nm. To overcome this limitation the electron microscopes were developed by Ruska and Knoll. Here the resolution is limited by the electrons wavelength, which is much lower than for visible light (100 000 times shorter). Both technologies are based on lenses that focalize a photon or an electron beam on a sample. Scanning probe microscopes work in a completely different way. The principle relies on the measure of a parameter (*e.g.* the tunneling current [1] or the force [2]) between a sharp tip and a surface and to keep this parameter constant while scanning in order to get a three dimensional image of the sample. As stated by C. Gerber, one of the Atomic Force Microscope (AFM) pioneer, and P. Lang in a 2006 paper in Nature Nanotechnology [3]: the scanning probe microscopes (SPM) have opened the door to the nanoworld. SPM made it possible to explore and to manipulate it. Feynman had dreamed of "the room at the bottom" [4]; SPM had opened the doors (for example, Eigler and Schweizer wrote the acronym IBM with Xe atoms [5,6]). Particularly, AFM has contributed to major advances in very different fields from fundamental physic and chemistry to information technologies, molecular electronic and spintronic. Since 25 years [7–9] AFM has emerged as a first interest characterization technology in life science. The number of research articles, published each year, in which AFM is used has increased exponentially since 1981. Fig. 1A presents this evolution. It must be noticed that the increase of studies on living cells is slow. This is probably due to difficulties inherent to biology and living cells.

AFM can be used in imaging modes like contact mode or oscillation mode as described in Fig. 1B. In these modes a sharp tip mounted on a cantilever is scanned over the sample surface. In contact mode, the cantilever deflection is kept constant in order to apply a constant force and to generate isoforce images of the surface. In oscillation mode, the cantilever is oscillating near to its resonance frequency and the amplitude

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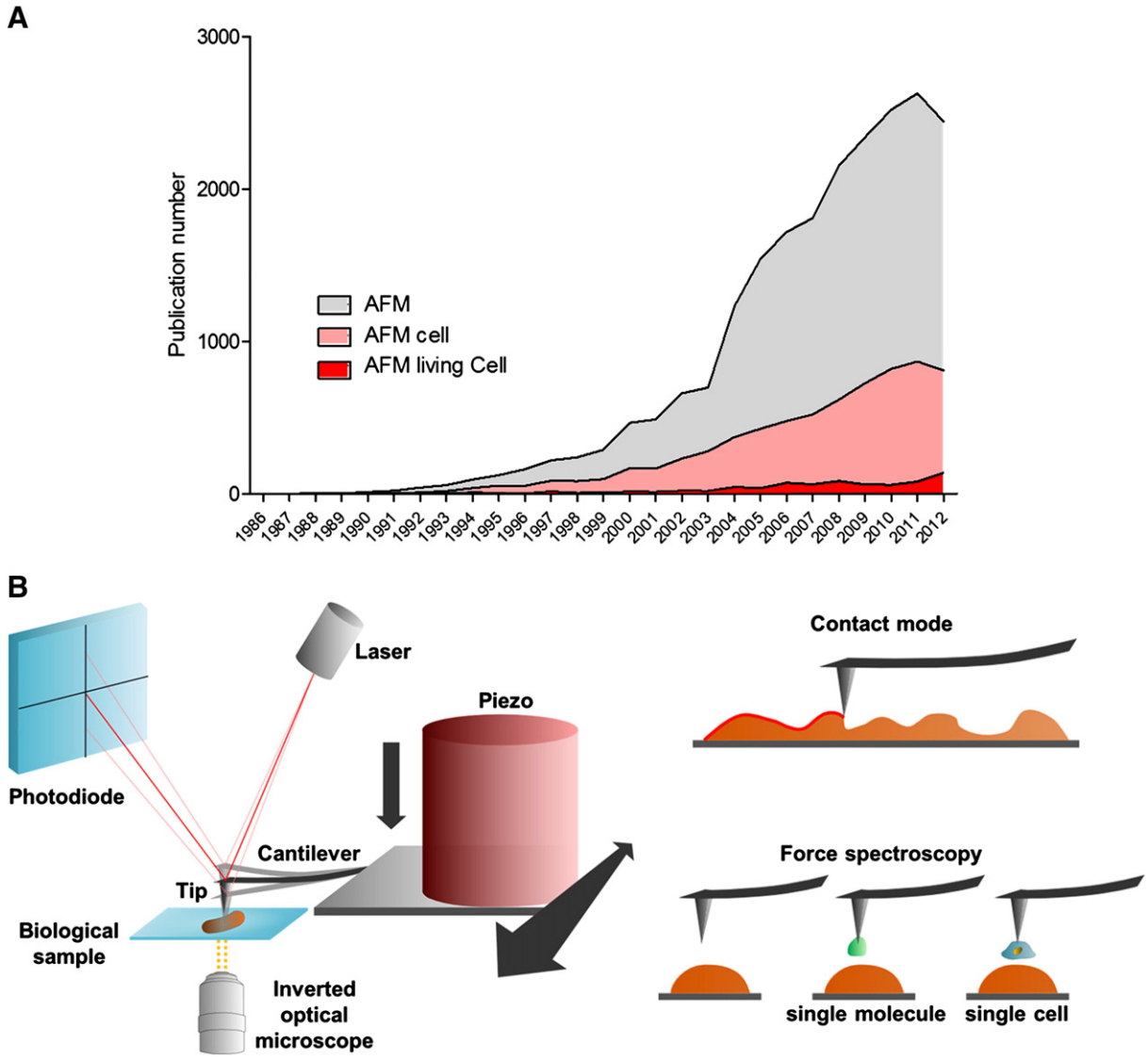


**Fig. 1.** (A) Evolution of the number of paper published each year, pubmed search using AFM or AFM and cell, or AFM and living and cell. (B) Schema introducing the AFM technology. A sharp tip is mounted on a cantilever that can be moved in the x, y, and z direction thanks to a piezo electric ceramic. The deflection of the cantilever is monitored on a 4 squares photodiode thanks to the reflection of a laser beam, aligned at the end of the, usually gold coated, cantielever. The AFM can be used to produce topographical images (like in contact mode) or to measure forces (in the force spectroscopy mode) between a bare or a functionnalized tip (with a biomolecule or a single cell) and the sample.

of the oscillation is kept constant while scanning, which creates isoamplitude images. However, AFM is not only an imaging technology. It is a highly sensitive force machine, able to measure forces as small as 10 to 20 pN. An AFM is therefore able to record force distance curves, which give measures and properties of the living material (this is sketched in Fig. 1). Nanomechanical properties and nano-adhesive properties of the samples can be measured using the AFM as a force machine. To make a link between the adhesive properties and a cell function it is possible to functionalize the AFM tip with a living cell. The results of such experiments create new paradigms in life science, and the interpretations in term of structure–function relationships are promising for pharmacologists. More and more articles are indeed dealing with the study of the effects of drugs on cells, studied by AFM.

The aim of our review is to give an overview of the AFM applications in biology (fungal cells, prokaryotic cells, mammal cells), with a special focus on the relevance in pharmacology. The first part is dedicated to fungal cells especially *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus fumigatus*. The second part treats of bacteria. It gives an insight on the fundamental knowledge that AFM has provided on bacteria and then emphasizes on studies dedicated to the study of antimicrobial (antibiotics, antimicrobial peptides, innovative molecules) effects. Finally, the third part addresses mammal cells, exposed to external stress, like drugs, but also diseases and cancer.

## 2. AFM for fungal cell wall analysis, from fundamental knowledge to pharmacology

Atomic Force Microscopy is a polyvalent tool that allows biological and mechanical studies of entire living microorganisms, and therefore the comprehension of molecular mechanisms. This first section introduces the AFM modes, with yeast cells as a eukaryotic model to illustrate its potentialities, and their implications in pharmacology. We will first explore morphological and mechanical studies on various yeast cells. Then, we will present molecular mapping principle on cell-wall surface and the applications of this technique for biology. Finally, we will investigate the yeast pathogenicity in cellular invasion and we will give an overview of AFM pharmacology's studies on yeast.

### *2.1. Morphological and mechanical studies*

Since its first development in 1986 by Binnig et al. [10], there have been an increasing number of AFM biological applications (Fig. 1). An

important part of publications corresponds to studies of cellular inner components and their mechanism of action. For example, different studies were dedicated to the visualization of nucleic acids like RNA or DNA with AFM [11,12] or protein oligomerization [13], but also biomolecular interactions such as Protein–DNA [14–16]. However, a major progress since 1995, made in AFM for biology offered the possibility to observe cells in liquid environment [17]. This first part will describe the AFM potentialities for high resolution imaging and probing the global nanomechanical properties of living cells.

*2.1.1. Imaging fungal cells*

As stated in the introduction, a main challenge is the gentle but firm immobilization of the biological sample required for any AFM experiment. Yeasts are round shaped cells of around 5 μm in diameter. The immobilization conditions have to maintain the yeasts in static position during AFM experiment. A solution described in the literature is to immobilize by drying the yeasts (Fig. 2A–B) [18,19]. This process causes cell death and the morphological properties of dead cells are different from living yeasts. To keep cells alive, AFM in liquid condition is required. And to this end, a rigorous, but non-denaturant immobilization method has to be used. A first AFM study in liquid condition was described by Gad and Ikai in 1995 [17]. The authors developed an immobilization method in agar surface to visualize living cells in native conditions; they observed bud scars for the first time by AFM. This example demonstrates the possibility to image living yeast, and thus structures at their surface that are directly linked to yeast-growth. More recently, porous membranes have been used to immobilize cells in liquid condition [20]. As shown in Fig. 2C, this method allows observation of bud scars on individual cells. In another study, the authors used this method to evaluate, at the nanoscale, the consequences of a defective cell wall in mutants yeasts, on the cell surface topography [21]. Finally, immobilization in PDMS stamps was developed. This method allows the immobilization of cells–yeasts (Fig. 2E) but also spores of *Aspergillus fumigatus* [22]. The mains advantages of this method are i) the transparency of the PDMS stamp which is therefore compatible with an inverted optical microscope, ii) the directed assembly of the cells, which result in predicted patterns of cells (no time is wasted to search for a cell), iii) the high number of cells trapped in the PDMS holes. The results presented in Fig. 2D shows the ultrastructure of the surface of spores of *A. fumigatus*; similar results were obtained with spores trapped in porous membranes [23]. Briefly, the spore is covered by a rodlet layer made of hydrophobins. These proteins self-organize at the spore surface, each rod being separated from its neighbor by 10 nm. During the spore germination, this nanostructure is disrupted.

Once immobilized, AFM experiments on yeasts can be conducted in different modes. The contact mode is an imaging mode. It consists in bringing a tip into contact with the surface, and scanning horizontally this surface with a constant applied force. An example of yeast imaged in contact mode was described in 1996 by Pereira et al. [24]. In this study, the authors observed different strains of *Saccharomyces cerevisiae*, the baker yeast, and showed that morphological aspects were different among strains. These observations revealed the high potential of contact mode to observe the morphological differences between yeasts strains. Another study performed in contact mode was dedicated to the visualization of different mutant yeasts defective in cell wall components; this study showed the involvement of cell wall architecture in the morphology of yeasts [21]. Finally, Kriznik et al. in 2005 characterized the morphological properties of the pathogen *Candida albicans* in its filamentous form but they used the taping mode [25].

In oscillation mode, stiff cantilevers are oscillated near their resonance frequency during the scan. The changes in the amplitude of oscillation report on the surface topography. Consequently, the lateral forces between the tip and the sample are reduced, which limits damaging of

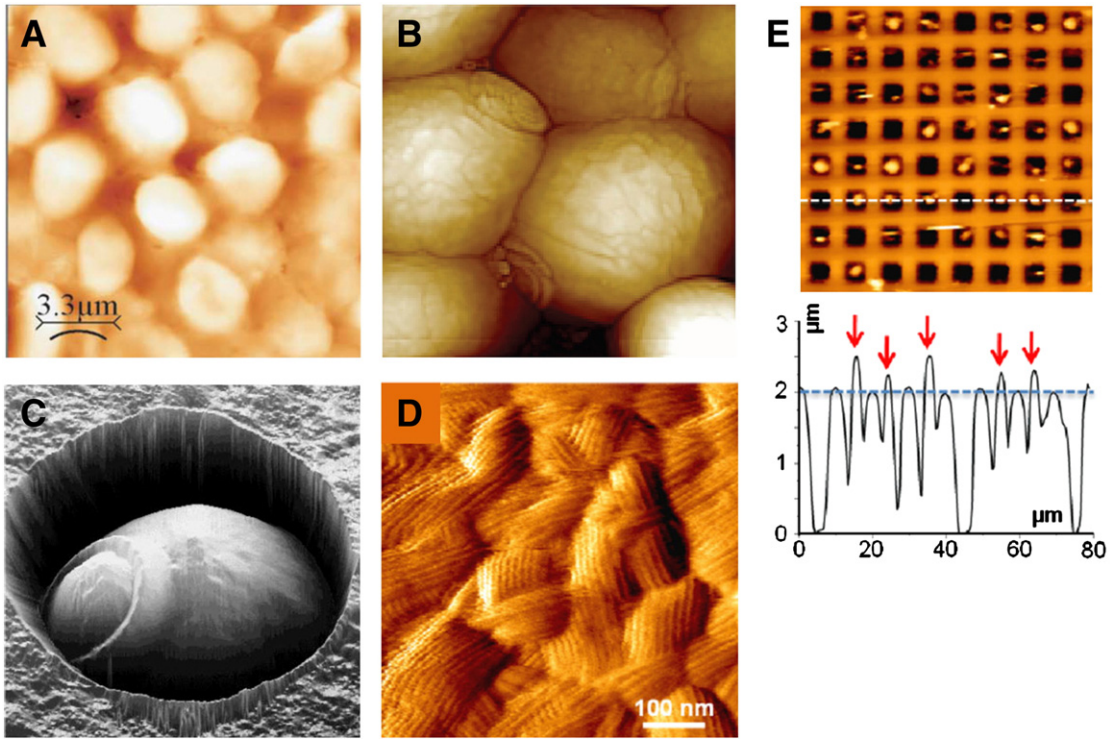


**Fig. 2.** High resolution imaging for yeast morphology studies. (A) Deflection image of dried *Saccharomyces cerevisiae*. The scale bar is 3.3 μm. (B) Height image of desiccated *S. cerevisiae*. The scan size image is 10 μm × 10 μm and the height scale is 0–4 μm. (C) Three-dimensional AFM height image (6 μm × 6 μm; z-range 1 μm), in aqueous solution, showing a single *S. cerevisiae* cell protruding from a porous membrane. (D) High-resolution AFM deflection image of the *A. fumigatus* spore surface. The scale bar is 100 nm. (E) AFM height images with associated sections of single *S. cerevisiae* yeasts trapped within the patterns of a PDMS stamp functionalized by ConA. Reprinted with permission from references [18–20] and [22] respectively.

the sample [26]. Several examples of oscillation mode images of yeasts can be found in the literature, to demonstrate morphological changes in cell-wall of *S. cerevisiae* according to various stresses [27] or to visualize rodlet structures on spores of *Aspergillus nidulans* [28].

### 2.1.2. Nanomechanical properties of yeast

In order to probe the nanomechanical properties of yeasts, such as spring constant, elasticity or turgor pressure, AFM is used in the force spectroscopy mode. In this mode the tip is continuously approached and retracted from the surface and force *versus* distance curves are recorded with spatial resolution. The approach curve describes the sample resistance to the applied force [29] and can be analyzed through theoretical physical models, giving access to mechanical parameters (Fig. 3A) [30]. When the tip is retracted from the sample, adhesion forces between the sample and the tip can be recorded, resulting in the measure of adhesion interactions. This will be described in the molecular mapping part.

To begin with, the spring constant of the sample can be deduced from an approach curve. To this end, the approach curve is fitted with the Hook model [31]. This spring constant describes the stiffness of the sample in N/m. Karreman et al., have for example measured the spring constant of yeasts grown with 0.8 M mannitol [32]. This induces an 8 time increase of the spring constant. The same behavior has been reported for yeast mutated for the gene HSP12. The defective cells also presented a high spring constant which has demonstrated the plasticizer role of the protein Hsp12.

However, the first nanometers of indentation, recorded on biological samples, are usually well fitted by the Hertz law [33]. By analyzing the data with this law, one can extract the Young Modulus value of the sample, meaning its elasticity in Pascal. The elasticity can reflect a cell state due to growth, environmental conditions or specific phenotype. Among others, it has been used to estimate the implication of certain genes in the yeast cell wall elasticity with different defective mutants [21,32], and to evaluate the influence of some molecules such as polyelectrolyte [34] or lithium [35] on the cell wall stiffness. However, it is to be noticed that living organism creates heterogeneous results. Indeed, differences can be observed on yeasts of the same strain in the same conditions (Fig. 3B) [22]. The important variations in YM reported on these five yeasts demonstrate a significant heterogeneity of cells coming from the same culture. Furthermore, YM values on the same cell are also heterogeneous, for example, the stiffness on a bud scar of *S. cerevisiae* is superior to the stiffness on another part of the cell wall, presumably due to an accumulation of chitin on the bud scar [36]. Similarly, Touhami et al. showed an increase of the stiffness in the regions of the yeast cell wall involved in the budding process [20]. Thus, one must be aware that repeatability is the key point to obtain values representing the whole sample elasticity. However, the resolution given by AFM allows measuring specific regions of interest and thus gives access to mechanical description of the surface of the cell.

Moreover, other mechanical properties than cell wall changes during growth-process can be described. An original work by Pelling et al. showed the change in the nanomechanical parameter of the cell-wall during motion of *S. cerevisiae* [29]. For this, the tip was put into contact with the cell wall and the cantilever oscillations were measured, translating cell-wall changes during motion. The authors were able to prove that a shift of temperature from 30 °C to 26 °C decreased the frequency of oscillation motion of cell-wall with similar amplitude. Furthermore, exposure of the cells to a metabolic inhibitor (sodium azide) caused the periodic motion to cease.

Altogether, nanomechanical measurements give new insights in the yeast cell wall organization and function.

### 2.1.3. Molecular mapping

Specific molecular interactions are the base of many biochemical processes. Recognition mechanisms involve several types of non-covalent bonds such as hydrogen bonds, Van der Waals forces, attractive/repulsive electrostatic and hydrophobic forces. The highly specific interactions between a ligand and its receptor for example, can be recorded by force spectroscopy during the retraction of the tip from the sample. To avoid the detection of non-specific events, the AFM tip can be functionalized with one of the actors of the interaction. These experiments, with functionalized AFM tips, are called Single Molecule Force Spectroscopy experiments, since they allow measuring specific interaction forces between only one molecule on the tip and one molecule at

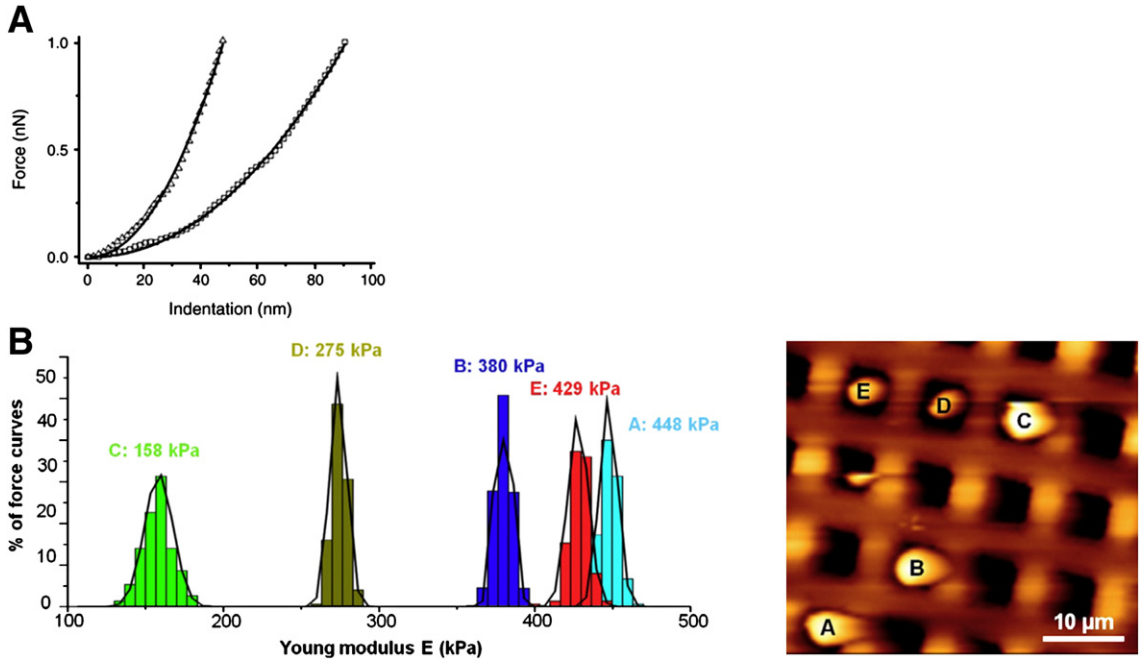


**Fig. 3.** Nanomechanical properties of living *S. cerevisiae* yeasts. (A) Force-indentation curves fitted by Hertzien model to extract a local Young modulus. The bud scar (triangles) has a modulus of 1.21 MPa, and the cell wall (circles) has a Young modulus of 0.54 MPa. (B) Young modulus determination on five *S. cerevisiae* yeasts. The histogram shows the Young modulus of each yeast trapped within the patterns of a PDMS stamp (AFM height image). Reprinted with permission from references [29,30] and [22] respectively.

the surface of the sample. In this part, we will first describe the molecular mapping principle. Then, different applications of molecular mapping on the yeast cell surface will be discussed.

### 2.1.4. Molecular mapping principle

As mentioned earlier, the tip functionalization is a prerequisite to measure specific interactions. Reviews of functionalization strategies can be found here [37–39]. A schematic representation of a tip functionalized with concanavalin A (protein that interacts with carbohydrates) is illustrated in Fig. 4A [40]. Adhesion forces were calculated from AFM retract force curves; an example of a retract force curve recorded during a single molecule force spectroscopy experiment is shown in Fig. 4B. The adhesion force was determined by measuring the piezo-retraction required to break the interaction between the lectin concanavalin A and the recognized carbohydrate. This process can be repeated several times, which enables, by moving the cantilever between each measurement, to obtain a map of the interactions. This indicates where the interaction is occurring, and thus how the probed molecule is distributed on the cell surface. In Fig. 4C, an adhesion map recorded with an AFM tip functionalized by concanavalin A, was obtained on a small region of a native yeast cell as indicated on the height image. Each pixel on the adhesion map represents a different force curve; the adhesion map gives therefore a global repartition of carbohydrates on the yeast-cell surface. The authors could conclude from these experiments that mannans were not uniformly distributed on the studied areas of the yeast cell wall [40].

### 2.1.5. Mapping and nanomechanical properties at the single molecule level

A recent example of molecular mapping study is the localization of Als3p on the yeast cell wall. Als3p is a protein (adhesin) involved in adhesion during host invasion. It was observed during the morphogenesis of *C. albicans* from yeast to hyphae [41]. This was performed with a tip functionalized by an antibody anti-Als3 (Fig. 5A). In the yeast form, adhesion maps show low rates of Als3 (Fig. 5B). However, during the hyphae transition, the Als3 rate is increased on the germinating yeast (Fig. 5C), and more specifically on the germ tube (Fig. 5D).

These changes were accompanied by a major increase of the hydrophobicity of the cell surface and confirmed the relationship between high adhesions in hyphae form and the pathogenicity of this form. In another study, the clustering of Wsc1, a transmembrane protein involved in stress response *via* the cell wall integrity pathway [42], was investigated on *S. cerevisiae* [43]. Molecular mapping was indirectly performed between Wsc1 modified by a Histidine tag, expressed by the yeast, and a tip functionalized by $Ni^{2+}$-nitriloacetate (NTA) groups. The authors proved that the clustering of Wsc1 was induced by stressing conditions, which suggested that this process was intimately connected to Cell Wall Integrity signaling pathway. This work confirmed that AFM was then a useful tool to understand molecular phenomena happening at the cell surface of yeast. Using the same methodology, another work demonstrated that cell wall thickness could be determined. To this end, different yeast mutants were generated, presenting increasing length of the Serine Threonine Rich (STR) region of the Wsc1 protein. This protein is anchored in the plasma membrane and is not detectable at the cell wall surface if the STR region is too short. The cell wall thickness was

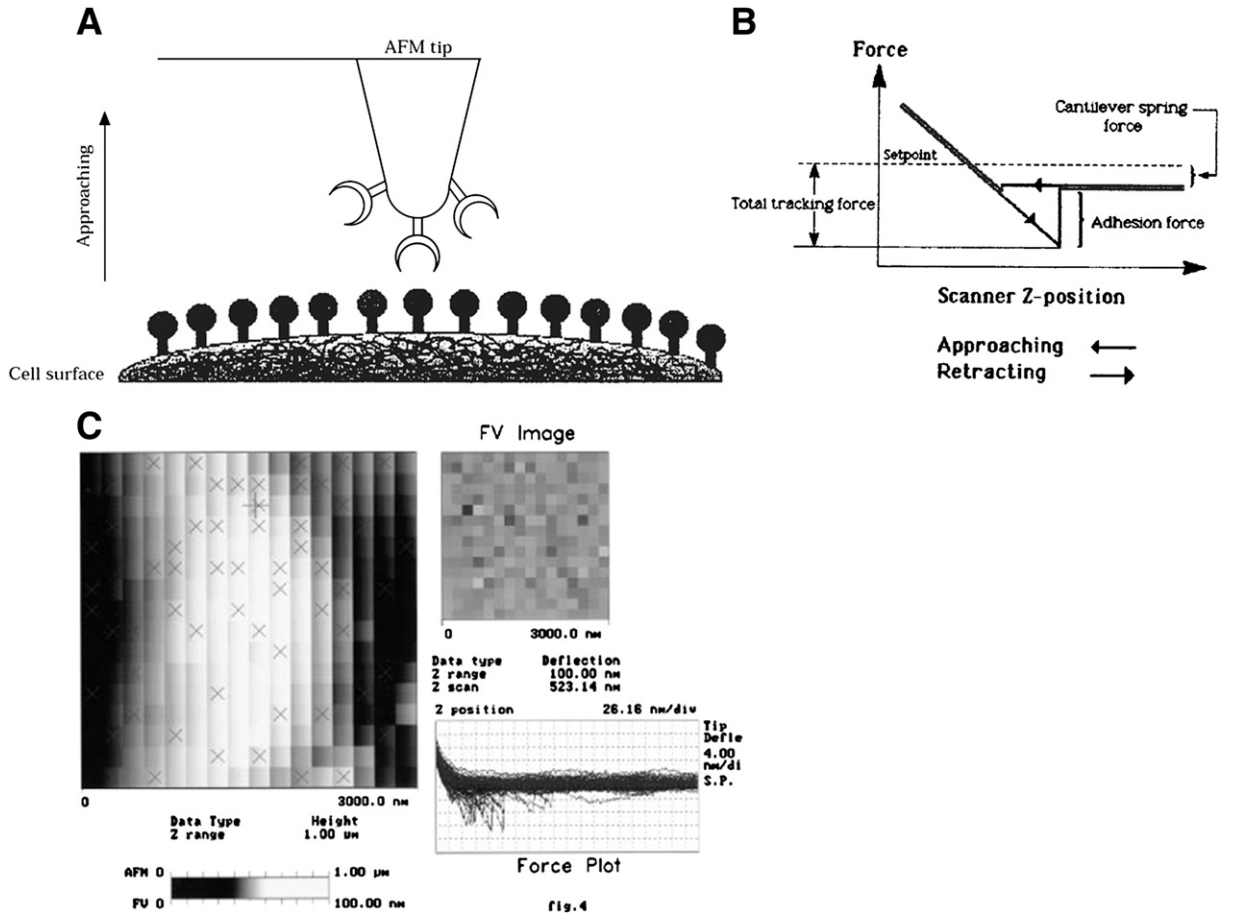


**Fig. 4.** Molecular mapping principle. (A) Model of experimental set-up configuration of molecular mapping by AFM. (B) Theoretical AFM force curve with adhesion force measurement during piezo retraction. (C) Typical force volume data frame showing different types of data that can be collected at the same time with this mode. Upper left is a height image, upper right is a force volume image, and lower right is a force curve display window. Reprinted with permission from Gad et al. [40].

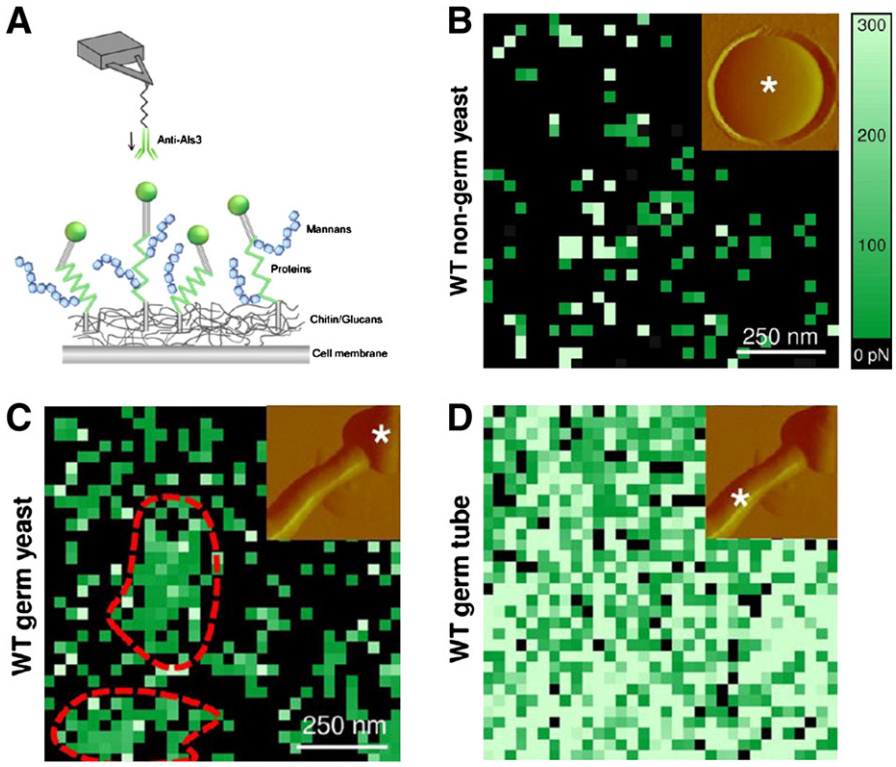


**Fig. 5.** Single molecular mapping according to *Candida albicans* morphology. (A) Schematic representation of interactions between Als adhesins (green) and anti-Als3 antibody immobilized on AFM tip. (B) Typical adhesion force map (1 μm × 1 μm, color scale: 300 pN) to probe Als3 proteins on yeast-form cell. Adhesion force maps for Als3 mapping on germinating yeast (C) and a germ tube (D). Reprinted with permission from Beaussart et al. [41].

thus deduced from the STR region length leading to the detection of Wsc1 at the cell wall surface [44]. AFM force spectroscopy measurements can also cause molecular re-organization at the surface of yeasts. For example, Alsteens et al. [45] demonstrated that the formation and propagation of the adhesin Als5 nanodomains in *C .albicans* was the consequence of localized delivery of piconewton force by the AFM tip functionalized with antibodies recognizing Als5. The same process was observed on dead cells, confirming that the process was not metabolic and indeed triggered by the AFM tip. The authors suggested that the functionalized tip could stretch, unfold Als5 and promote the aggregation and self-association of Als5. This process could be involved in cellular adhesion, in response to mechanical stimuli. In another study, with a tip functionalized with concanavalin A, difference in mannoproteins elongation was investigated between the cell wall surface of *S. cerevisiae* and *S. carlsbergensis* [36]. These experiments showed that only mannan chains were stretched from the surface of *S. carlsbergensis* whereas the entire mannoproteins were stretched from *S. cerevisiae*.

### 2.2. Yeast pathogenicity and pharmacologic studies by AFM

The number of fungal and yeast species on earth is around 611 000 [46]. Among them, only 600 species are human pathogens [47], like *A. fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum* or *C. albicans*. The last is one of the most common cause of hospital-acquired systemic infections, due to its adhesive and invasive properties, and its capability to form biofilms [48]. In order to fight against this pathogen, AFM has been used to study its virulence mechanisms, and to understand the effects induced by antifungal treatments on its cell wall [49,50]. This part will first focus on host–pathogen interactions involving *C. albicans*, studied by AFM. We will then describe the most recent studies on the effects of antifungal drugs on the cell wall of yeast.

#### 2.2.1. Understanding yeast interactive behavior

For pathogens, adhesive properties are fundamental for host-invasion or biofilm formation. A first study by Götzinger et al. quantified the yeast *S. cerevisiae* adhesion to surface of silica particles by AFM [51]. The yeasts were immobilized on an AFM tip functionalized with concanavalin A, which interacts with the carbohydrates present at the surface of yeasts. Authors proved that adhesion of yeast to silica particles was very variable according to the pH solution and the roughness of silica particles.

More recently, interactions between *S. aureus* and *C. albicans* were investigated by AFM [52,53]. These pathogens are classically found in combination during human tissue infection. To understand their relationship during infection, the authors quantified the interactions between *S. aureus* immobilized on an AFM tip, and different regions of *C. albicans*, in yeast and hyphae form including three parts, the head, the middle and the tip (Fig. 6A). Adhesion forces were quantified during piezo retraction at the initial contact (0 s) or after 60 s of bond-maturation. The results in Fig. 6B demonstrate that *S. aureus* interacted preferentially on the hyphae form (tip and middle) and hardly on the head part or on the yeast form. Furthermore, the authors made the hypothesis that the 60 s of contact were required for adhesion because an active reorganization of the hyphae cell wall was used by the yeast to promote the adhesion of *S. aureus*. These observations confirmed that during infection *S. aureus* was interacting only with the hyphae form *of C. albicans*.

A direct AFM observation of the interaction between *C. albicans* and macrophages was presented by El Kirat et al. [54]. This study showed by differential interference contrast (DIC), fluorescence and AFM, the main steps of macrophage infection by *C. albicans*, including initial intercellular contact, internalization of yeast cells, intracellular hyphal growth and pathogen externalization from the macrophage. An example is given in Fig. 6C, where we can see yeast internalization into the macrophage. The ability to directly visualize these biological processes

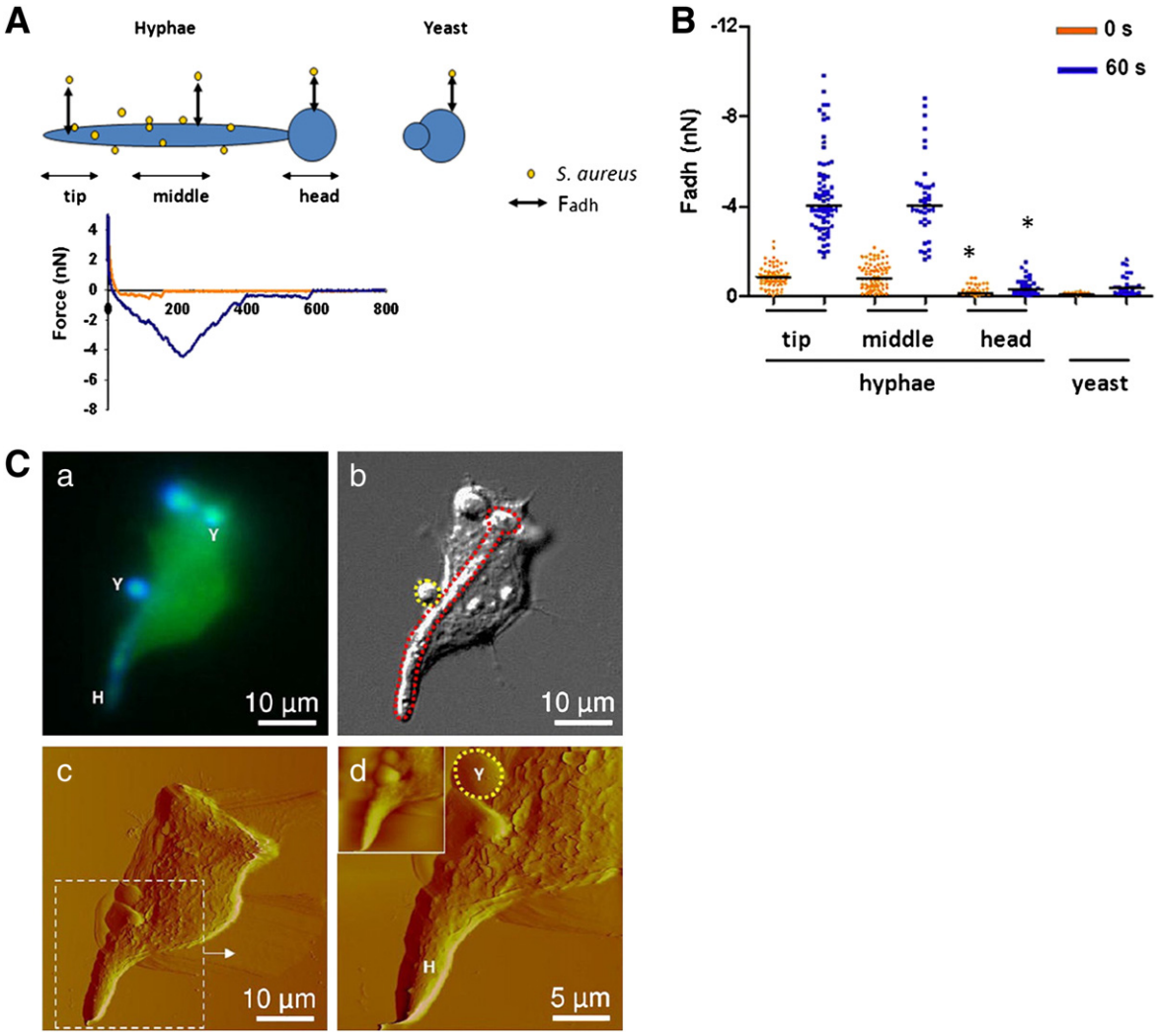


**Fig. 6.** Yeast–cells interactions. (A) At the top, schematic representation of the different hyphal regions defined for adhesion between *Staphylococcus aureus* and *Candida albicans hyphae*. At the bottom, example of force distance curves between *S. aureus* upon initial contact (orange curve) and after 60 s bond-maturation (blue curve) with *C. albicans* hyphal tip region. (B) Vertical scatter bars adhesion forces between *S. aureus* and different *C. albicans* morphologies. (C) Imaging of a single macrophage infected by *C. albicans* visualized by fluorescence (a), DIC (b) and AFM deflection images (c,d). Labels Y and H correspond to yeast and hyphal cells. The red and yellow dashed lines in (b) indicate an internalized hyphal cell and a free yeast cell, respectively. Reprinted with permission from references [52] and [54] respectively.

demonstrates how AFM can be used to understand infection mechanisms and help in anti-fungal investigation.

*2.2.2. Pharmacology studies*

AFM is also a promising tool to characterize the antifungal molecules effects on the morphology and the nanomechanical properties of the yeast cell wall. However, AFM pharmacology studies concerning yeast are very limited and only few studies were performed so far. For example, the ultrastructure alteration of the cell wall of *S. cerevisiae* by the tetrapisispora phaffii killer toxin (Kpkt) was characterized by AFM [55]. The authors demonstrated that Kpkt caused an alteration of the cell wall with a specific β-glucanase activity. In another study, AFM was used to evaluate the cell wall roughness of *C. albicans* after lemon grass oil (LGO) treatment in vapor phase [56]. The authors observed a decrease in the roughness of the cell wall. AFM was also used in another study to describe the biophysical properties associated with cell death

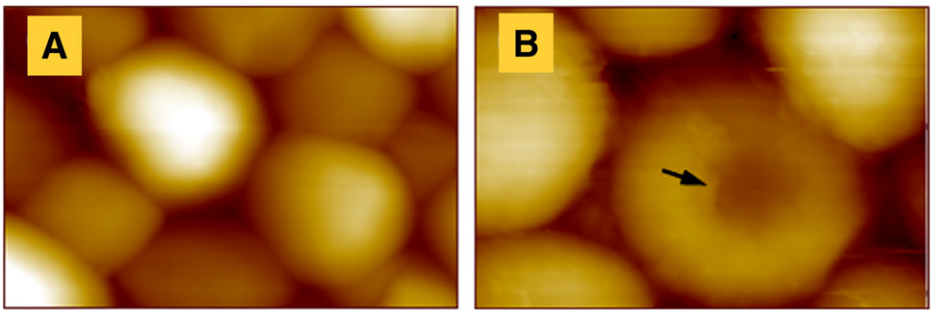


**Fig. 7.** Anti-fungal effect of allincin. AFM images of dried *C. albicans* untreated (A) or treated (B) during 24 h with allicin and amphotericin B. The arrow indicates a significant morphological change. Reprinted with permission from Kim et al. [50].

due to flucytosine (an analog of fluorinated pyrimidine, which mainly acts on RNA and DNA) and amphotericin B (acts on cell walls by an unknown mechanism). After drug treatment, the cell wall of *C. albicans* was perforated, deformed, and shrunken [49]. However, in contrary with LGO, the roughness of the cell wall was increased. In addition, a combination between drug treatment, such as allicin (organic compound harmful in yeast cell growth) and amphotericin B decreased the yeast viability and induced significant cell wall damages (burst or collapsed membranes) as shown in Fig. 7 [50].

These examples show how antifungal drugs induce cellular damages and death of pathogenic yeasts. Furthermore, they confirmed the potentiality of AFM for pharmacological studies. In addition, the AFM development during the last decades with others biological systems, such as bacterial and mammals cells, opens the way in pharmacology for microbiology and cancerology.

## 3. Atomic Force Microscopy in bacteriology

Atomic Force Microscopy has proven itself to be a powerful tool for the study of microbial systems. In this section, we will explore the applications of AFM to the bacterial field. In one hand, we will give an update of the studies dedicated to the morphology and behavior of bacteria alone, or interacting with their environment. Then, in another hand, we will focus on pharmacological studies that have been performed on bacteria, with antibiotics and antimicrobial peptides, as well as with innovative molecules.

### 3.1. Fundamental bacteriology

Bacteria are ubiquitous on earth, and have negative side effects in many fields such as food industry or human health. They can also be used to our benefit such as in waste water treatment plans or pharmacy (production of recombinant drugs). Bacteria interact with their environment through their surface and a lot of researches are therefore focused on the microorganism's surface. In this context, Atomic Force Microscopy has become more important, and an increasing number of researchers have exploited both imaging and force measurements capabilities to explore bacterial surface in terms of structure and function.

#### 3.1.1. Imaging bacterial morphology

One of the most common applications of AFM in microbiology is to visualize the morphology of microorganisms. AFM provides the opportunity to image single bacterial cells; it can also be used to image several cells, as it is the case in biofilms for example, where aggregates of microorganisms adhere to each other on a surface [57]. Many bacteria are characterized by their shapes (coccus, bacilli or spores) and by their nanoscale ultrastrucutres, for example S-layers, capsular polysaccharides, flagella or fimbria. Fimbriae, also known as pili, are thin, hairlike appendages on the surface of gram-negative and gram-positive bacteria, that perform a variety of different functions such as genetic transfer *via* conjugation, movement across surfaces, and adherence to a variety of surfaces [58]. Scanning Electron Microscopy (SEM) has long been the only tool available for the direct observation of bacterial pili; however, this technique does not allow any quantitative analysis. With the recent progress made in biological application, it is then naturally that researchers interested in bacterial pili turned to AFM, such as for example Schäffer's team that investigated the geometric and elastic properties of the pili of different *Corynebacterium diphtheriae* strains, the etiological agent of diphtheria [59]. Their measurements showed that among mean-visible contour-length of the pili, there were significant strain-specific differences that could not be correlated to the efficiency of adhesion to substrates. In another study by Touhami et al., [58], the morphology of *Pseudomonas aeruginosa* pili were investigated using AFM, and the authors also studied the ability of pili to adhere to mica surfaces, highlighting the role of pili in bacterial adhesion. Finally in another study, Dufrêne's team successfully visualized self-assembled nanostructures that are formed by the pili of *Lactobacillus rhamnosus* GG [60].

Flagella differ from pili in their proteic composition. The bacterium flagella is a sophisticated molecular nanomachine composed of three substructures that provide motility, anchor the structure into the cell membrane, and a last one that acts as the rotary motor [61]. Flagella contribute to the virulence of pathogenic bacteria, and AFM imaging is a powerful tool to analyze the morphology of the flagellum. Different studies report on the morphology at high resolution of flagella; Gillis et al. have studied the expression of flagella in relation to its function, and showed that for different strains of *Bacillus thuringiensis* exhibiting different levels of flagellation, the amount of flagella observed at the nanoscale could be correlated with the motility behavior of the strains [62]. In another study conducted by Diaz et al., the authors used AFM imaging to determine if the growth of flagella was oriented during the early stages of biofilms formation [63]. The authors showed that it is indeed the case; the flagella are first oriented towards the neighboring cells, making contact and finally surrounding them. Chang et al. were more interested in the morphological modifications induced by pH on the flagella of *Escherichia coli*; they showed that both acidification and alkalization of the culture media was affecting the morphology of the flagella, by reducing theirs diameters [64].

Another nanoscopic ultrastructure that characterizes bacteria is the capsule, made of polysaccharides. Capsular polysaccharide has important functions for bacteria as for example nutrient uptake, protection against environmental stresses, adhesion to different surfaces, or survival against phagocytosis or antibiotics. This structure is therefore a virulence factor of the bacteria able to produce it, and AFM imaging has enable to study the morphology of this capsule and understand its surface characteristics. Among the studies dedicated to the capsule of bacteria [65,66], is the work conducted by Suo et al. in 2007. The authors show that HEPES, a buffer commonly used in biological experiments and presumed to have no effects on specimens, stabilizes the capsule formation of *E. coli* and *Salmonella typhimirium* [67]. An example on *S. typhimirium* is given in Fig. 8A; we can see on this amplitude image an aggregate of cells covered by capsular polysaccharide, after the cells were rinsed in HEPES buffer. Coldren et al. in 2009 worked on *S. aureus* capsule and could understand, using AFM, how the capsular polysaccharide is important for bacterial adhesion to surfaces, and therefore for biofilm formation [68].

Bacteria that do not display capsular polysaccharide can however display bacterial surface layers (S-layers). S-layers are 2D-cristalline arrays of glycoproteins; they represent the most common cell surface structures in bacteria [69]. As for capsular polysaccharide, S-layers are the frontier between the cell and the environment; they therefore play several roles such as protecting the cells from environmental stresses, or for nutrient uptake. Because of the particular self-assembly of S-layers proteins, it has been a good model for AFM imaging at high-resolution. S-layers proteins can either be recrystallized on surfaces such as silicon [70] or gold surface [71], or directly studied *in vivo* as Dupres et al. did [69]. In this study the authors imaged nanoarrays of S-layers on live *Corynebacterium glutamicum* and observed hexagonal unit cells with dimensions similar to those reported on isolated S-layers sheets. Their work also led to the discovering of a new inner layer composed of periodic nanogrooves, which could probably reflect the specificity of the *C. glutamicum* cell wall.

Bacteria can grow in bulk or form biofilms; some bacteria can also form endospores. Endospores are the disseminating agent of bacteria; their formation is triggered by conditions of limited nutrient availability and environmental stress. They are highly resistant to extreme temperatures or chemicals. The transformation of a dormant spore into a vegetative cell is an important step in the pathogenicity of the bacteria, and can be imaged using AFM. A few studies were dedicated to the germination of spores of *Bacillus anthracis* [72], or *Bacillus atrophaeus* [73,74]. One is a technical jewel since it shows the complete high-resolution imaging structural dynamics of single spores

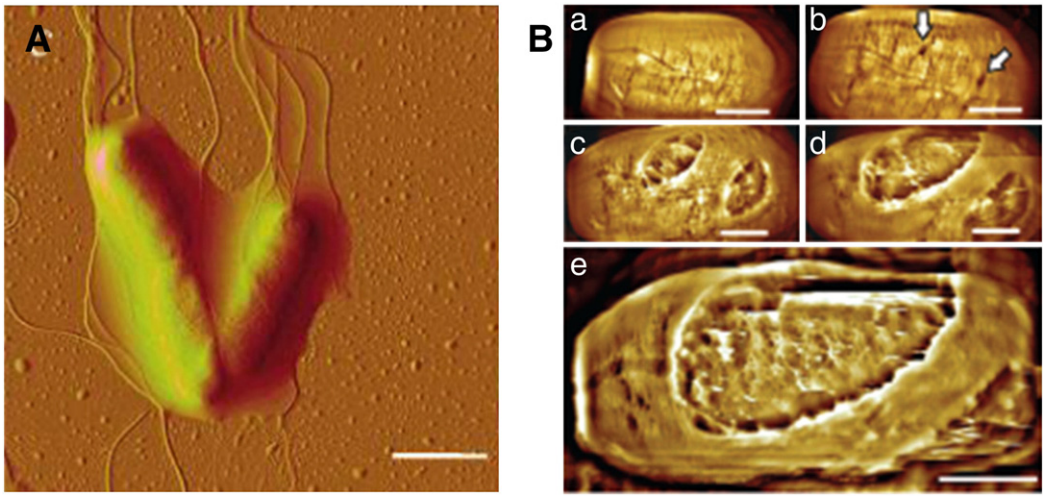


**Fig. 8.** High resolution imaging bacteria. (A) AFM amplitude image of aggregate of three *Salmonella typhimirium* cells covered by an EPS capsule. Note that part of the EPS is confined by the flagella. Scale bar = 2 μm. (B) Series of AFM height images showing the emergence of vegetative cells of *Bacillus atrophaeus*. The images show 60- to 70-nm-deep apertures in the rodlet layer (indicated with arrows in b), and subsequent eroded the entire spore coat (e). Images in a–e where recorded on the same spore; elapsed germination time (in hr:min) was as follows: (a) 3:40, (b) 5:45, (c) 7:05, (d) 7:30 and (e) 7:45. Scale bar = 500 nm. Reprinted with permission from references [67] and [75] respectively.

of *B. atrophaeus* [75]. In this study, the authors imaged the emergence of vegetative cells (Fig. 8B) trough rodlets apertures at the surface of the spore. These images are not only impressive for the phenomena they show, the also give new insights into the structure of native peptidoglycan; the fibrous network observed on Fig. 8B–C on the germ cell surface seems to represent nascent peptidoglycan architecture of newly formed cell wall. The authors then found a good experimental model for investigating the genesis of the bacterial peptidoglycan structure.

### 3.1.2. Nanomechanical properties of bacteria

The mechanical properties of bacterial cells, such as elastic moduli, spring constant or turgor pressure, can be investigated by AFM, *via* nanoindentations measurements. We will focus in this paragraph on elastic properties of bacterial cells that are expressed by Young's modulus. Understanding the elasticity of cells is important for elucidating the mechanisms underlying cells growth and behavior in different conditions. It is also known that, in many living organisms, the mechanical properties of the external membrane can indicate the state of the underlying system. In this frame, many studies using nanoindentations were used to study the bacterial cell wall. For example, Francius et al. found that the elastic properties of bacterial cells of *E. coli* were dependent on the expression of surface appendages such as fimbriae, and also on the ionic strength of the medium they are grown in [76]. Schaer-Zamaretti et al., however, could distinguish between different strains of *Lactobacillus* expressing or not S-layers by probing the elastic properties of such cells [77]. Gaboriaud et al. used nanoindentations measurements to understand the influence of a different pH on the nanomechanical properties of bacterial cells [78]. But a major concern in bacteriology is to determine whether a bacterium is dead or alive. That is the question that Cerf et al. answered with the nanoindentations measurements; as it is shown in Fig. 9A, live cells of *E. coli* show a Young modulus of

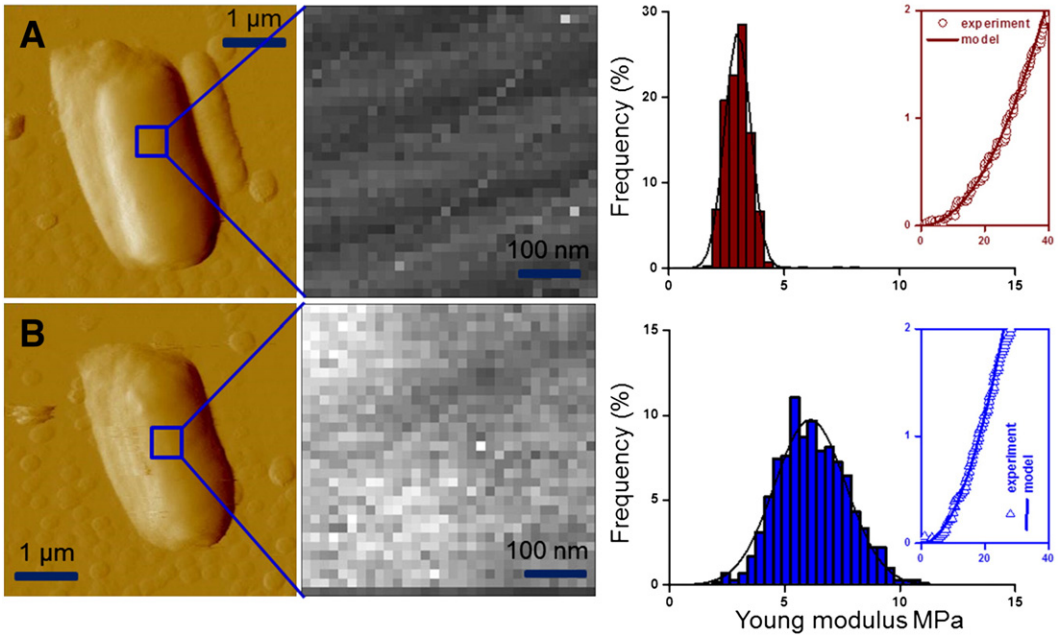


**Fig. 9.** Nanomechanical properties of bacterial cells. (A) Nanomechanical properties of alive *Escherichia coli* cells. AFM deflection images of single living *E. coli* bacterium and (B) of the same single bacteria killed by thermal treatment (20 min, 45 °C). The elasticity maps (z-range = 10 MPa) correspond to the images insets. The elasticity distribution is represented by the histograms, together with a typical force curve. Reprinted with permission from reference [79].

3.0 MPa, whereas heated (dead) cells (Fig. 9B) have a Young modulus increased to 6.1 MPa [79]. The authors then demonstrated that dead cells with a damaged membrane exhibit higher Young modulus values. A recent novel development in analysis of force curves generated in nanoindentations measurements allow now to extract the elastic modulus at the cell surface, but also in depths under the cell surface [80]. Longo et al. used this new analysis development to probe the nanomechanical properties of *E. coli* cells [81]. In this study, the authors found stiffer areas at the surface of the cells, and when they go deeper into the cells, they find these stiffer areas to have a complex form, that changes during time. This could be correlated to an accumulation of complex molecules underneath the cell membrane and reorganizing in the cell cytoplasm over time.

### 3.1.3. Probing molecules at the interface of bacteria

Besides imaging and probing nanomechanical properties of bacteria, it is also possible with AFM to study molecules at the surface of bacteria with bare or functionalized AFM tip. Understanding how complex molecules are assembled at the surface of bacteria under physiological conditions is of fundamental importance to elucidate their functions in different processes such as cell aggregation, adhesion to substrate, or interactions with external molecules or organisms. A first example is the interactions between bacteriophages and bacteria. Dubrovin et al. proposed two articles where the authors investigate the interactions between bacterial cells and bacteriophages [82,83]. While the first study focuses on the characterization of phages, and the effects of phages interactions with different strains of bacteria, the second study is dedicated to the investigation of the lytic cycle of the *Acinetobacter baumannii* bacteriophage AP22.

The results presented in Fig. 10A present AFM images of *A. baumannii* cells infected by AP22 bacteriophage over time. After 1 min of interaction between bacteria and phages (Fig. 10Aa), the phages have adsorbed on the surface of the cells; the high resolution image (Fig. 10Ab) clearly shows phage heads. However, after 3 min of incubation (Fig. 10Ac and d) the phages heads are empty, leading to the supposition that DNA release from the head takes place quite fast, and probably immediately upon phages adsorption on the cell's surface. And this phenomenon is even more visible after 6 min of incubation (Fig.10 Ae and f). Also others studies dedicated to bacteria/external molecule interactions were performed [84], and among them is one focusing on the interactions of different strains of *Lactococcus lactis* with mucins [85,86]. In this study, the authors, using cell probes, were able to measure the kinetic association/dissociation constants between the bacteria and the mucins.

The interactions with other microorganisms are mediated *via* surface molecules of bacteria. One of these molecules at the surface of gram-positive bacteria is peptidoglycan, a complex polymer made up of glycan strands of repeating disaccharides residues, cross-linked *via* peptide side chains. This molecule is a vital molecule for bacteria, as it is responsible for shape determination and cellular viability, and since it is a target for a lot of antibacterial treatments, it has been extensively studied. However few AFM studies report on the structure of peptidoglycan [87–89], and among them is the one of Hayhurst et al., [90], in which the authors propose an architectural model for the peptidoglycan of *Bacillus subtilis*. As it is shown in Fig. 10B, the authors claim that glycan strands form a "rope" that is coiled into a helix; this rope runs then all over the bacteria.

For gram-negative bacteria, the molecules that can be found on their surface are polysaccharides and lipopolysaccharides (LPS). Francius et al. in 2008 studied the localization and conformation of single polysaccharides at the surface of live *Lactobacillus rhamnosus* GG using functionalized AFM tips [91]. Using concanavaline A, a lectin that interacts with carbohydrates, he could pull off the surface individual mannose. As we can see on Fig. 10C, on a mutant strain impaired in adherence to epithelium, there is a dramatic decrease in adhesion

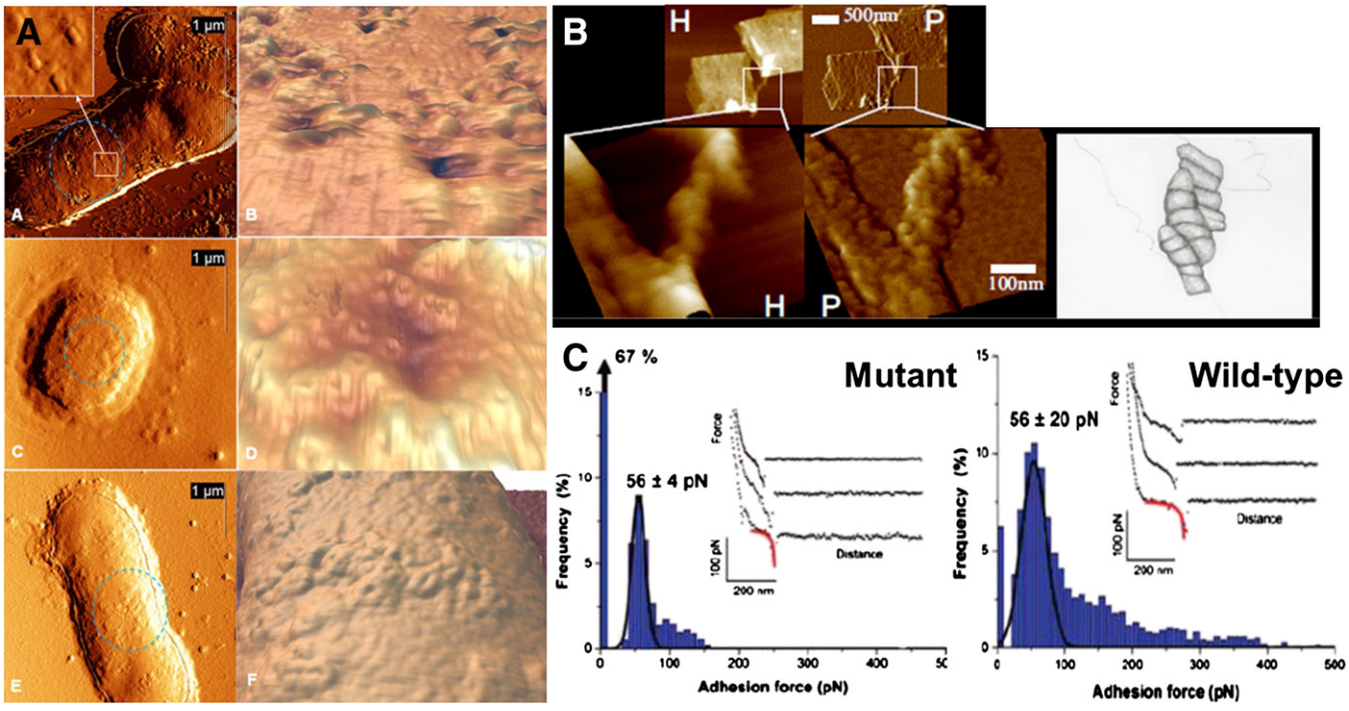


**Fig. 10.** Mapping and interactions with molecules at the interface. (A) Bacteriophage interactions with *Acinetobacter baumannii*. AFM images of *A. baumannii* cells infected for 1–6 min by the bacteriophage AP22. Left row: deflection AFM images of *A. baumannii* cells incubated with bacteriophages AP22 for 1 (a), 3(c), 6 (e) minutes. Right row: zoomed-in three dimensional reconstructions of bacterial surfaces depicted on the left (the zoomed regions are indicated by dotted circles in the height images). The inset in (a) demonstrates zoomed-in region, shown by the white square. (B) *Bacillus subtilis* sacculi architectural features revealed by AFM. The images show two cylinder fragments, from a purified sacculi from gently broken cells, joined by a twisted cable. H stands for Height image and P for Phase image. (C) Detecting individual mannose-rich polysaccharides on LGG bacteria. Adhesion force histograms are shown, (n = 1024) together with representative force curves recorded in buffered solution with a Con A tip on LGG wild-type and on the mutant CMPG5413 (impaired in adherence to gut epithelium, biofilm formation, and exopolysaccharide production). Reprinted with permission from references [83,90] and [91] respectively.

frequency compared to the native strain. Therefore, this technique of Single-Molecule Force Spectroscopy can be used to understand which molecules interacts with, in this case, the gut epithelium. Another study by Strauss et al., studied the role of LPS O-antigen on the adhesion of *E. coli* cells, using also AFM, with bare AFM tips [92], but there were also works performed on the coat proteins of spores of *Bacillus* [93,94], and on the adhesins at the surface of living mycobacteria [95].

### 3.2. Evaluating the effects of antibacterials molecules by AFM

Understanding the nanoscale behavior of bacteria under physiological conditions, or their interactions with other organisms in their native state is a first point; we need to know the "enemy" to efficiently fight against them. However, the common way to fight against bacteria is to use antibacterials. Among them, there are the antibiotics, used at home or in hospitals to treat infectious diseases, but there are also other kind of antibacterials, less used or under development, such as antibacterial peptides, and innovative molecules, that should be known about. This section will focus on how AFM techniques (imaging, force spectroscopy, single molecule force spectroscopy) can help understanding the nanoscale effects of antibiotics, or understanding the mechanism of action of new molecules not yet fully characterized.

#### 3.2.1. Nanoscale effects of antibiotics on bacteria

Antibiotics have in most cases a well described mechanism of action on bacteria [96]. However, the effects on bacterial surface at the nanoscale are poorly understood, and for this purpose, the AFM technique is particularly well suited, since it allows imaging and probing nanomechanical properties on single living cells. Among antibiotics investigated by AFM, examples are β-lactams [97,98], aminoglycosides and their derivate molecules [99] and fluoroquinolones [100]. The results presented in Fig. 11A shows morphology modification of *P. aeruginosa* treated by ticarcillin (β-lactams) and tobramycin (aminoglycoside) [97]. As we can see, ticarcillin causes an elongation of the cells, whereas tobramycin alters the surface of the cell. But antibiotics cause also modifications of the cell wall nanomechanical properties, such as elasticity and spring constant. Francius et al. probed on living *S. aureus* cells the effects of lysostaphin, an enzyme that cleaves the peptidoglycan, over time [101]. The authors found that the lysostaphin treatment caused a decrease in the elasticity of the cell wall with the time of treatment, along with a decrease of the spring constant of the cells. These modifications could only be probed with force spectroscopy, and give precious information on the nanomechanical properties modification that antibiotics cause on bacterial cells.

Other authors were interested in vancomycin, a glycopeptide antibiotic, used in last chance in hospitals. This molecule binds with high affinity and specificity to the terminal D-Ala-D-Ala peptidoglycan precursors, leading eventually to cell lysis. In the study of Gilbert et al., AFM tips were functionalized with vancomycin, and used to perform single molecule force spectroscopy experiments on *L. lactis* during the course of division [102]. The results obtained (Fig. 11C) show that the D-Ala-D-Ala residues are located on the division septum of the cell, which suggest that the newly formed peptidoglycan is inserted in this region during the division process. This study demonstrates that AFM with antibiotic-modified AFM tip is a valuable tool to explore the dynamics of antibiotic–ligand interactions; it also gave new insights on the assembly process of peptidoglycan in gram-positive bacteria. Antimycobacterials have also been a subject of interest, and AFM investigations have been performed on *Mycobacterium JLS* [103] and *Mycobacterium bovis* BCG [104].

#### 3.2.2. Antimicrobial peptides

However, bacteria becoming more and more resistant to antibiotics, new approaches have to be developed to find new ways of killing bacteria. Antimicrobial peptides have been developed for several years, but for now, their mechanism of action is not fully understood and need further studies. To this aim AFM can be used to evaluate the effects of such

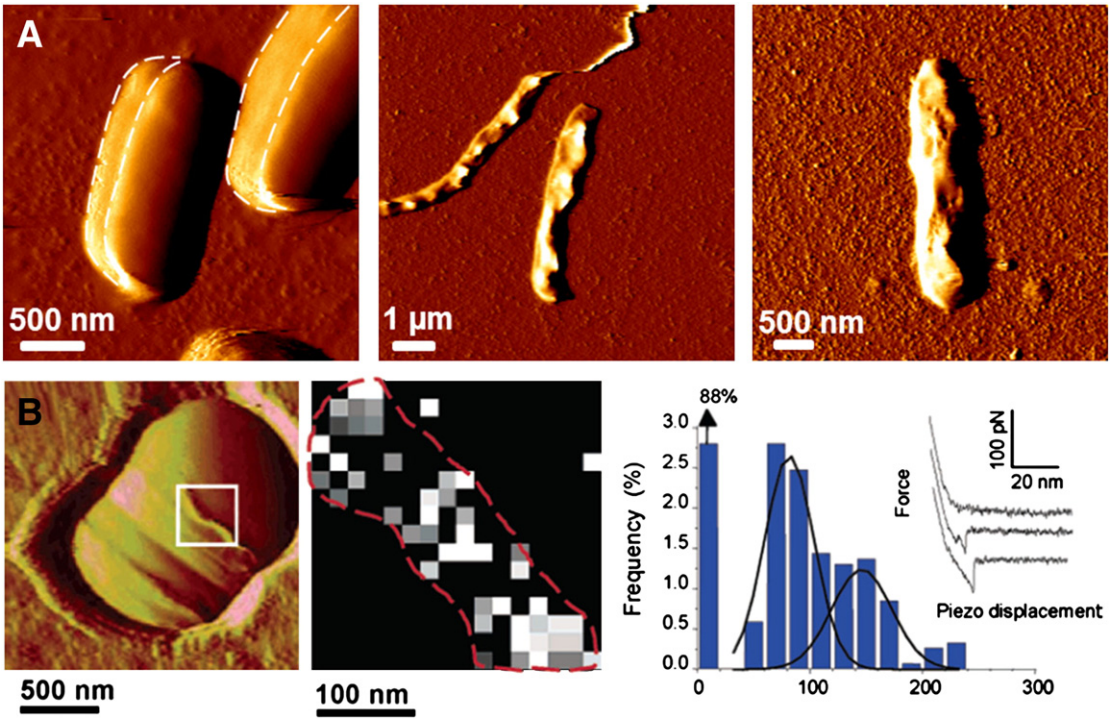


**Fig. 11.** Effects of antibiotics on bacteria. (A) Effects of ticarcillin and tobramycin on *Pseudomonas aeruginosa*. Vertical deflection images of native live cells of *Pseudomonas aeruginosa* (left image) treated by ticarcillin (middle image) and tobramycin (right image). (B) Imaging individual D-Ala-D-Ala sites on living *Lactococcus lactis*. The AFM image shows a single wild-type *Lactococcus lactis* cell during the course of the division process. The cell is located at the center of the image and trapped into a porous polymer membrane for noninvasive, *in-situ* imaging. The middle image represents the affinity map (gray scale: 100 pN), together with the adhesion force histogram (n = 1536), and representative force curves recorded with a vancomycin tip on the septum region (highlighted by the white box in the AFM image), using constant retraction speed (1000 nm/s) and interaction time (500 ms). Reprinted with permission from references [97] and [102] respectively.

molecules at the nanoscale, helping to the understanding on how these peptides interact with the bacterial cell wall. The most famous of antimicrobial peptide is colistin; this peptide is efficient against gram-negative bacteria, such as *Acinetobacter baumannii*, *P. aeruginosa* or *Klebsiella pneumoniae*, bacterial strains that are the cause of many cases of nosocomial infections. Very few AFM studies were dedicated to the study of the effects of colistin on gram-negative bacteria [105–107]; the main conclusions coming from these reports are that treated bacteria have an increased cell wall elasticity and spring constant, along with a decrease in the adhesive properties of the cells. These conclusions are consistent with the hypothesis that colistin acts as a detergent on the bacterial cell wall and removes the LPS from the surface. In fact, in these studies, results are also presented with colistin-resistant bacteria, that have cell wall structural differences compared to susceptible strains.

Research in the antimicrobial peptide field is actually emerging and many papers treat of the evaluation of the effects of various original peptides with AFM. The peptides studied have diverse origins; for example PGLa peptide comes from frog skin and its secretion and has been showed to remove the outer membrane of *E. coli* while decreasing the surface stiffness [108]. Meincken et al. compared the effects of three different peptides, melittin from the honeybee *Apis mellifera*, magainin and PGLa from frogs skin [109] on *E. coli*. While these peptides are close in terms of amino sequence, the authors could make the difference between the effects caused by each one of them on bacteria, thanks to AFM. Finally Li et al. evaluated the effects of sushi peptides S3 on *E. coli* cells [110]. The results presented in this paper show that after 1 h of treatment, bacteria are severely damaged and large amounts of cytoplasmic fluids are exuded. This indicates drastic permeabilization of the inner membrane, which would be the second step of the S3 mechanism of action. The first one is a damaging of the outer membrane; the second is the initiation of the permeabilization of the outer membrane, and finally the release of the cytoplasmic fluid from the bacteria. Therefore, the authors, thanks to AFM, could get a better understanding of the mechanism of action of a novel and still unknown antimicrobial peptide.

And since antimicrobial peptides are supposed to have a detergent-like action on the membranes of bacteria, many studies focused on characterizing the effects of peptides on phospholipidic layers that mimic these membranes. Roes et al. used reconstituted monolayers of LPS from *Salmonella enterica* to study the effects of polymyxin B; Francius et al. observed the interactions between supported bilayers of DOPC/DPPC and surfactins [111]. As a last example, Arseneault et al. made DPPG mono- and bilayers to understand the interactions with lactoferricin B, an antimicrobial peptide obtained from the pepsin cleavage of lactoferrin [112].

#### 3.2.3. Innovative molecules and nanotechnologies

Whereas antimicrobial peptides have a mechanism of action different from antibiotics, still resistances to colistin, for example, have started to emerge. The research must therefore explore new possibilities for finding new antibacterial molecules that are different from both antibiotics and peptides, in order to avoid development of resistances. A new approach developed is the one of nanoparticles. Metallic nanoparticles are promising antibacterial agents since they are chemically stable, resistant to heat and have a long life. Currently a broad variety of metals and their compounds are used in microbiology research for their potential antimicrobial activity. There are already a few studies reporting on AFM investigations of the effects of nanoparticles on bacteria [113–115], and one of them is dedicated to copper iodide nanoparticles [116]. In this study, the authors have synthesized and evaluated the effects of this novel kind of nanoparticles on different bacterial strains, including multidrug resistant ones. The results of this study show that for *E. coli* (K12) cells treated with the nanoparticles, the membrane is totally disrupted compared to the native cells. In addition, the authors were also able to prove that copper iodide nanoparticles caused the generation of reactive oxygen species, therefore damaging the DNA of the bacterial cells.

Another new approach developed in the recent years is the one of calixarene molecules. An example of such molecule is the paraguanidinoethylcalix [4]arene (Cx1), that has been proven to be efficient on both gram-negative and gram-positive bacteria [117]. However the mechanism of action of this molecule was still unclear, and AFM techniques were used to get a better understanding of this molecule interaction with the cell wall of bacteria [118]. The results presented in Fig. 12A show that on a multidrug resistant strain of *P. aeruginosa*, Cx1 causes a dramatic decrease of the cell wall elasticity, from 517 to 75 kPa. This information shows that Cx1 is efficient on such a bacterial strain, and that its action destabilizes the cell wall of the gram-negative bacteria. To go further into the mechanism of actions, the authors probed the cell wall of treated and untreated bacteria with an AFM tip functionalized with concanavalin A, a lectin that binds to sugars. These single molecule force spectroscopy experiments revealed that the lectin could unfold a molecule only on Cx1 treated cells (Fig. 12B). This molecule could possibly be the peptidoglycan, which would be accessible because of the destabilization of the outer membrane caused by the interaction with Cx1. Research is still going on in this field, and new calixaneric molecules are under development [119–121].

Along with nanoparticles and calixarene, there are also approaches that involves carbon nanotubes which have been proven to be efficient against *E. coli* and *B. subtilis* [122], and chitosans, that were investigated using AFM on *B. cereus*, *E. coli* and *S. aureus* [123,124]. Eventually, emerging area of interest is the use of probiotics to cure infections. As the use of lactobacilli and staphylococci for the treatment of vaginal infection, that has been studied by cell–cell interaction [125] or cell–protein interaction as for lactococcus and mucin in the case of gastrointestinal infection [85,86] and probiotic action [126].

To conclude, we show in this section that Atomic Force Microscopy opens new perspectives for characterizing bacterial species, and understanding the molecular and nanomechanical processes underlying their behavior in physiological conditions. We have also seen that AFM techniques are very useful to study the nanoscale effects of antibiotics that have a known mechanism of action. However, since bacteria are becoming more and more resistant to the antibiotics, new approaches involving innovative molecules are developed and once again, AFM can be used to get a better understanding of the mechanism of action of these new molecules, with the hope that they will be used to treat nosocomial infections caused by multidrug-resistant bacteria.

## 4. AFM for mammal cells pharmacology's studies

This part focuses on the insights that Atomic Force Microscopy can give in the field of pharmacology for mammal's cells, from the general understanding of therapeutics treatment to special cases of diseases, with a whole part concerning cancer study. Most of the studies in this field are performed on cells lines or patient isolated cells *in vitro*, but recent progress have made possible to study entire biopsies, thus enlarging the use of AFM. It is to be noted that AFM studies of mammal cells are increasingly performed in combination with fluorescence microscopy. Coupling of these two techniques offers a wide range of possibility in studying biological phenomena. We will concentrate mainly on AFM results, but keep in mind that fluorescence microscopy and AFM are complementary in this type of studies.

### 4.1. General applications

The use of Atomic Force Microscopy for pharmacology's studies on mammalian cells have been led by 2 types of research field: 1) the understanding of cell response to an external stress (infection, injury) characterized by immune and differentiation or gene expression response; 2) the investigation of drug effect on cells, from internalization efficiency to affectation of cellular processes and active effect on pathogens. The following section will be then divided in 2 parts related to these concerns.

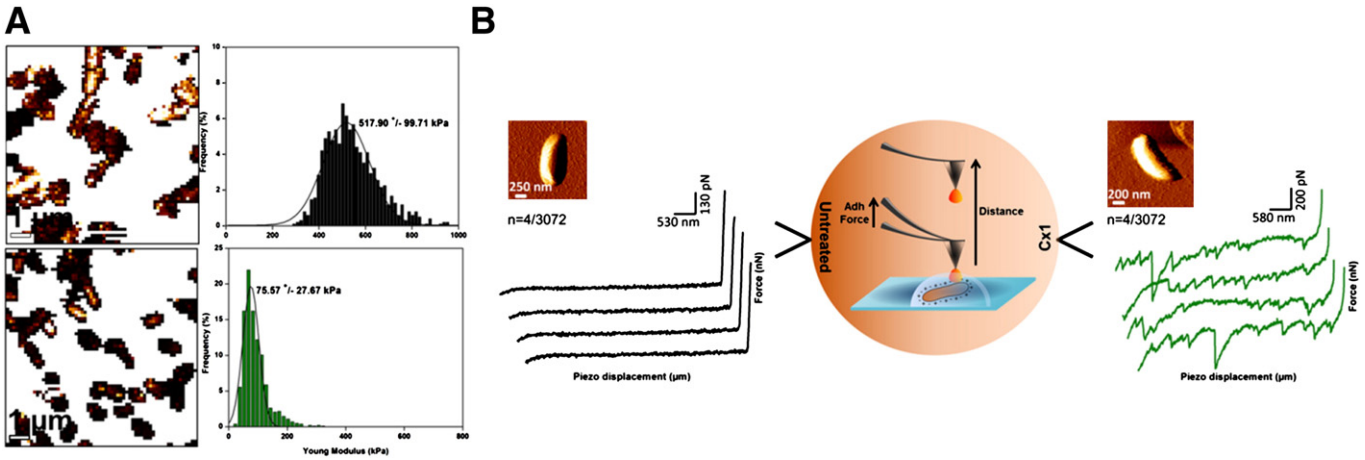


**Fig. 12.** Effects of innovative antibacterial molecules on bacteria. (A) Antibacterial effects of calixarene Cx1 on the elasticity of *Pseudomonas aeruginosa* multidrug resistant. Elasticity map (z-range = 1.5 MPa) of several cells of untreated *P. aeruginosa*, together with distribution of young modulus values (top images), and of cells treated with Cx1 (bottom images). (B) Effects of Cx1 on the architecture of the cell wall of *Pseudomonas aeruginosa* multidrug resistant. Schematic representation of the force curves (retract segment) obtained with ConcanavalinA functionalized AFM tips on untreated *P. aeruginosa* cells (left curves) and Cx1 treated cells (right curves). The 4 force curves (n) presented by conditions were chosen out of 3072 curves recorded on 3 different bacteria coming from 3 independent cultures. Reprinted with permission from reference [118].

*4.1.1. Cell response to external stress*

In the complex context of multicellular organisms, the cell response to an external stress is of interest to accurately target the dysfunction in case of disease. For example, the study of keratinocyte reaction to surfactant by AFM can help in the understanding of chemical stresses on the skin, showing that despite a morphological effect, no differences in stiffness are measured at non cytotoxic doses of sodium lauryl sulphate [127]. But AFM can also help in immune response characterization. Immune response is based on first, the detection of pathogen and then, their elimination. On one hand, lymphocytes B are involved in the humoral immune response, and recognize molecular component in extra cellular fluids that directly inform of the pathogen presence in the organism. These cells are responsible for antibody production. Their activation is a complex mechanism that appeared to change

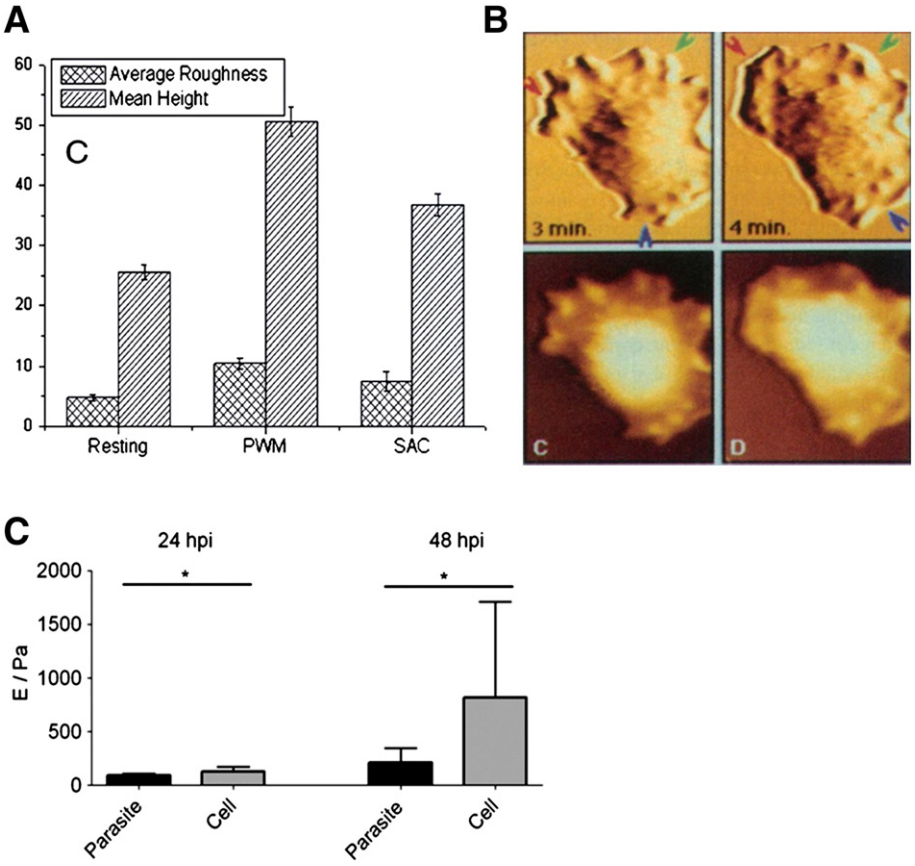


**Fig. 13.** AFM in pharmacology. (A) The average roughness and mean height particles of surface nanostructure of resting and B lymphocytes after their activation by pokeweed mitogen (PWM) or *Staphylococcus aureus Cowan I* (SAC). Mean ± SD. (B) AFM imaging of platelet activation along time, height image (z-range of 0–1.9 µm) and corrugation images (z-range of 0–280 nm). (C) Increase in stiffness of human hepatoma cells infected by *Plasmodium flaciparum*. ($P < 0.05$). Reprinted with permission from references [132,152,216].

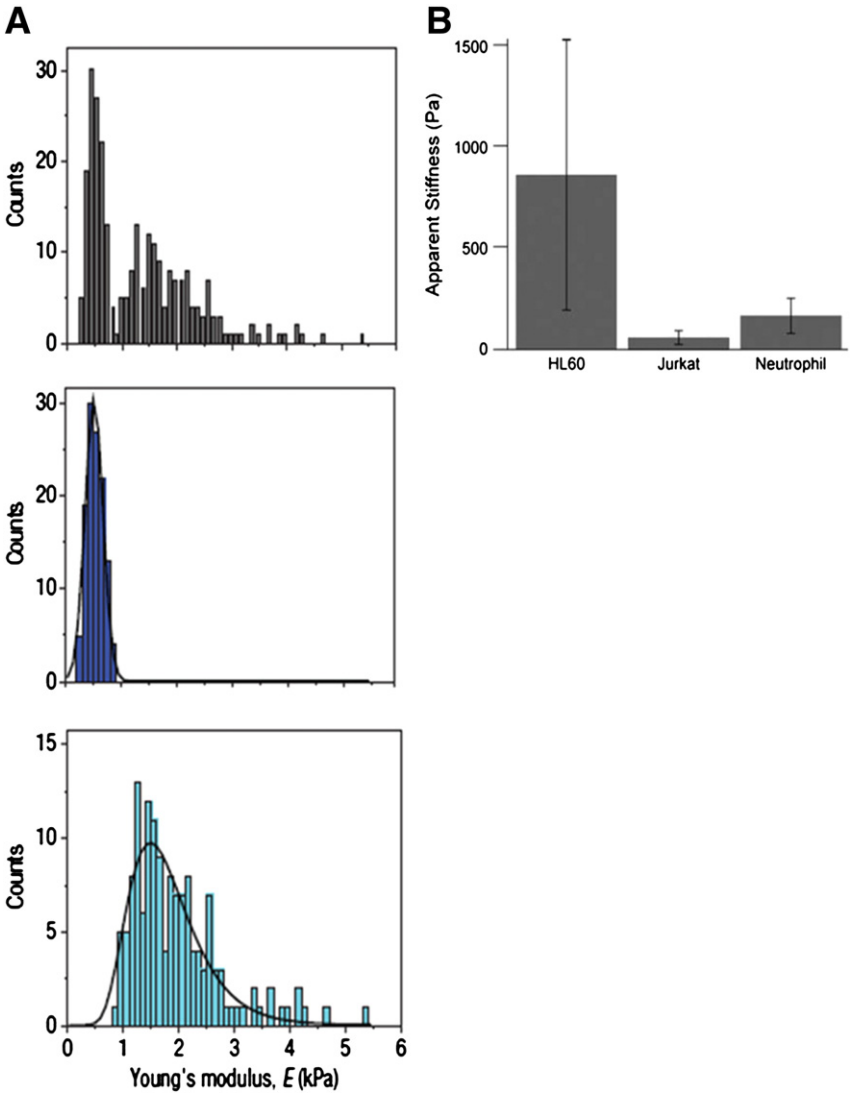


**Fig. 14.** Cancer cell characteristics. (A) Increase in cell elasticity of leukemic cells (HL60) leading to leukostasis. (B) Elasticity of cancer cells collected from patients with suspected metastatic cancer from seven different clinical samples. Grey: measurement for all cells together. Dark blue: cancer cells. Light blue: normal cells. Reprinted with permission from references [157,158].

their nanomechanics. Wang et al. have shown, using AFM, that activation of B cells by pokeweed mitogen (PWM) or *S. aureus* Cowan I (SAC) induces the clustering of B cell receptors at the cell surface, responsible for cytoplasm biochemical activation cascade and improvement of cell adhesion to antigen. This clustering is visible by topographic analysis such as roughness and particle (cluster) size (Fig. 14-A). Cell adhesion capacities have also been evaluated by force spectroscopy [128]. On the other hand, Lymphocyte T cells are responsible for cell-mediated immune response. To achieve their goals, these cells need to reach the site of infection (inflammation site), and then to cross endothelial barriers, which involves cell–cell adhesion, namely lymphocyte T–vascular endothelial cell adhesion. This crossing implies a change in adhesion protein expression pattern, like integrin and selectin, by endothelial cells. Zhang et al. have investigated the adhesion dynamics of the T-cell–endothelial interaction by force spectroscopy study between a functionalized tip where a lymphocyte cell is attached and endothelial cell on the substrate [129]. The adhesion forces have been measured and discriminated using antibodies against major adhesion proteins expressed by endothelial cells. These experiments lead to the conclusion that these proteins are the ones involved in lymphocyte B adhesion to endothelial cell. This inflammatory process involves also the increase of temperature, and thus heat stress. HSP60 protein (heat shock protein) production by endothelial cells has been studied by single molecule force spectroscopy and revealed the presence of this protein at the membrane surface of heat stressed cells and its possible implication in arthrogenesis [130]. Finally, macrophages phagocytosis plays an important role in the elimination of pathogens and dead cells following immune response [131].

As another stress that an organism is subjected to, injury is one of the most common one. In the process of maintaining blood vessel integrity, platelet activation is the first and key process. Topographical analysis of platelets activation has shown cytoskeleton reorganization at a resolution of 50 nm. A redistribution of the platelets granula and vesicles towards the lamellipodia of the cell has also been observed; this phenomenon leading to increase plasma membrane surface thus improving aggregation (Fig. 13B) [132].

We can see through these different examples that most of cellular responses involving different gene expression pattern are linked to cell membrane reorganization and changes in nanomechanical properties of the cell. These processes are observed for other mechanisms like cell differentiation or pathway activation. Han et al., in 2011 have validated the IGF-II (insulin-like growth factor) autocrine signaling pathway as a suitable target for the detection of muscle differentiation using AFM. They have been able to discriminate differentiated cells from others by

the detection of IGF-II at the cell surface thanks to anti-mouse IGF-II antibody immobilized on the AFM tip [133]. Using the same concept, Qiu et al. have characterized quantitatively the TRA-1–81 (un-differentiation marker) expression level on Human Embryonic stem cells surface to capture the "turn-on" signal and understand the mechanism of their early differentiation [134]. Another way to discriminate differentiated cell can be to measure their elasticity, as it is the case for the change from osteoblast (stiff) to osteocytes (soft) [135]. This stiffness is directly correlated to the capacity to adhere to substrate, and is accurate to test the biocompatibility of implant materials [136].

*4.1.2. Drug effects and disease studies*

Drug effect investigation can be done by the three classical measurements: adhesion force, elasticity or imaging, as for example this study about the effects of an anti-malaria compound on leukemic cells [137] or hormonal effects that have been widely studied and well documented by Hillebrand et al. [138]. i) Single molecule force spectroscopy has resolved at the molecular resolution the dynamics of AMPAR neuron receptors trafficking under NMDA stimulation [139]. ii) Effects of asthma drugs (aminophylline) on red blood cell elasticity have been correlated to their reduced capacity to transport oxygen in capillaries by increasing their stiffness and thus reverse the drug purpose [140]. iii) These elasticity changes are linked to a change in cell shape too. Imaging of cells leads to the understanding of molecule side effect, as for example chlorpromazine. Li et al. have shown that this schizophrenia drug affects endothelial cell morphologically [141]. $HgCl_2$ toxic effects have been also characterized by imaging cells in contact with the molecule [142]. Finally imaging can also be used to study the mechanism of drug internalization, as for example the study of the direct DNA insertion thanks to the AFM tip for nanomedicine [143].

We will now focus on the special case of cardio-vascular affections. Indeed, cardiac cells (cardiomyocytes) present the distinctive feature of being contractile, and AFM is nevertheless technically of interest to study them, as shown by Liu et al. This group has measured cell contractility with or without incubating them with Ibutilide, a classical drug used to treat arrhythmia, and started to determine its cellular target and mechanism of action [144]. The last development allows synchronizing AFM measurements with the contractility of the cells, providing the possibility to detect specific events [145]. The evaluation of recovery after infarct by elasticity measurements showed that stroma cell-derived factor 1α (SDF-1α) increases the elasticity of peri-infart mice tissue border zone and stiffening the scar, thus conducting to a better resistance to ventricular remodeling and infarct expansion [146]. The importance of the protein Ephrin B1 in the lateral membrane of cardiomyocytes has also been assessed by AFM. The deletion of Ephrin B1 makes the cardiomyocytes stiffer and progressively leads to the cardiac tissue disorganization. This protein is essential for the stability of cardiac tissue architecture cohesion [147]. Nevertheless, beyond the whole cell study, AFM can be run on organelles like mitochondria, which dysfunction is known to be implicated in the pathology of myocardial infarction. The release of cytochrome C by mitochondria was correlated to its swelling by fluorescence microscopy, thus not quantitatively. Lee et al. have shown that mitochondria indeed swell during apoptosis; the authors also gave quantitative morphological

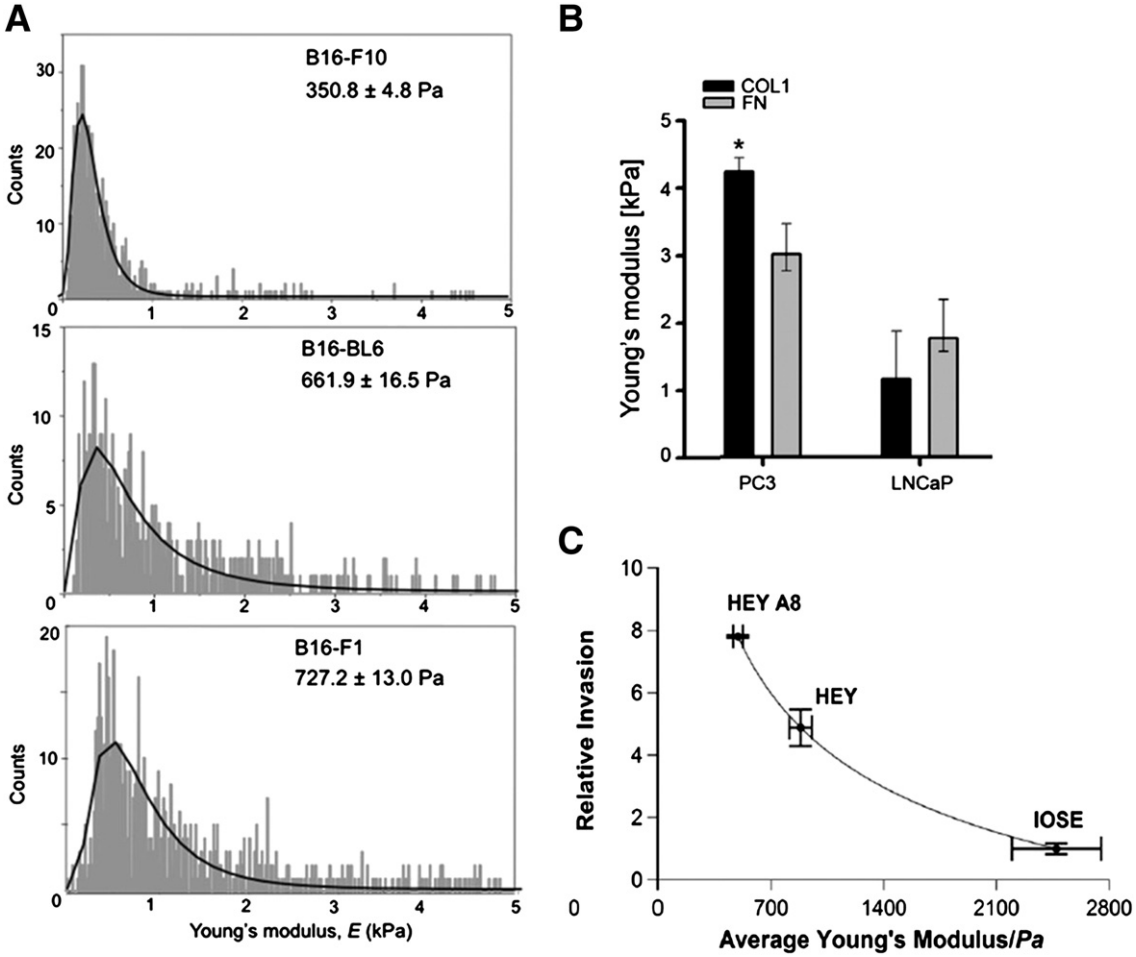


**Fig. 15.** Relation between stiffness and metastatic state. (A) Young's modulus distribution of murine melanoma cell of decreasing metastatic potential (B16-F10, B16-BL6 and B16-F1cells). (B) Substrate related elasticity of PC3 and LNCaP cell when culture on collagen (COL1) or fibronectin (FN). (C) Invasion *versus* average stiffness for ovarian surface epithelial cells (IOSE), and ovarian cancer (HEY and HEY A8 cells are HEY A8 cells that are more tumorigenic). Reprinted with permission from references [161,164,168].

analysis of rat heart mitochondria ischemic or not in native conditions. Furthermore, AFM allows them to detect nano-mechanical surface properties changes of ischemic cell mitochondria that are linked to their swelling [148].

Vascular diseases are also subjected to investigation by AFM. The inner part of blood vessel is responsible for blood pressure regulation through different mechanism like the release of vasoactive substance namely nitric oxide. The release of nitric oxide by the inner endothelial cells have been shown to be correlated with (and maybe regulated by) cell cortex stiffness changes [149]. Artherosclerosis lesions are affecting arterial cells stiffness too, specifically in branches and curved region of blood vessel, where endothelial cells appear to be stiffer than others endothelial cells [150]. This may be related to oxidized low-density lipoproteins (ox-LDL) cell exposure [151].

Furthermore, AFM can give answers to the questions raised by the pathogen infection process. For example the *Plasmodium falciparum* liver infection can be characterized by an increase in stiffness associated to a cell response to infection (and not the presence of the microorganism itself) (Fig. 13C), and may further enhance the understanding of this clinically silent step [152].

### 4.2. A tool for cancer-study development

Considered as the disease of the 20th century, cancer remains one of the most complicated and unsolved disease, meaning that no recovery treatment exists and only the eradication of tumor cells shows consistent results. In the following section accent will be put on the progress AFM allows for understanding this disease and the consequent medical applications that emerge with this nano-mechanical tool.

#### 4.2.1. Nanomechanic of cancer cell

In 1999, Lekka et al. have for the first time compared the elasticity of cancerous cells *versus* "normal" ones [153]. They studied human epithelial bladder cells lines and show that cancerous cells present a lowest young modulus value than normal cells. This article was part of a set investigating several cancer types : prostate cancer cells [154], breast cancer cells [155] or cervical ones [156], all showing a decrease in stiffness for cancer cells, except in the case of leukemic cells where cell stiffness increase leading to leukostasis (Fig. 15A) [157]. In 2007, the same observations were published on cells directly taken from the body (pleural) fluids of patients with suspected lung, breast and pancreas cancer [158], validating the decrease of elasticity as a characteristic of cancerous cells, *in vitro* and *in vivo* (Fig. 15B).

These observations lead the authors to wonder why there was this particular change in elasticity and so to investigate the mechanics of the cancer cells. Rapidly the idea of the involvement of cytoskeleton in this measured softness was pointed out [159] and studied [160]. Moreover, the changes in elasticity of melanoma cell lines have shown that the decrease in stiffness was directly correlated to the metastatic potential of the cells [161], the stiffness being the lowest for melanoma B16-F10 cells that produce a large number of foci, and the highest with a reduce number of foci (Fig. 16A). The same relation has been shown for oesophageal cells lines in different phases of premalignancy [162].

All together, these data demonstrate that the nanomechanical properties of cancerous cells are linked to their condition, which enable them to change their elastic properties (deformability) in order to cross the cells barriers to create metastasis in the organism (leave the primary site, pass into circulation, stop at a secondary site and migrate again across the vascular barrier). Beyond this metastatic state, cancerous cells must be able to sense their environment to efficiently invade the right targeted-tissue. Firstly, the Young modulus of cancer cells was shown to change when cultured on different substrates, indicating a perception of their environment, and yet different capacities depending on their "function" [153]. For example elasticity of breast cancer cells decreases on fibronectin [163], which is related to the cancerous nature of the cells as shown on prostate cancer cells *versus* non-cancerous cells cultured on collagen (Fig. 16B) [164]. The extra cellular matrix is thus of

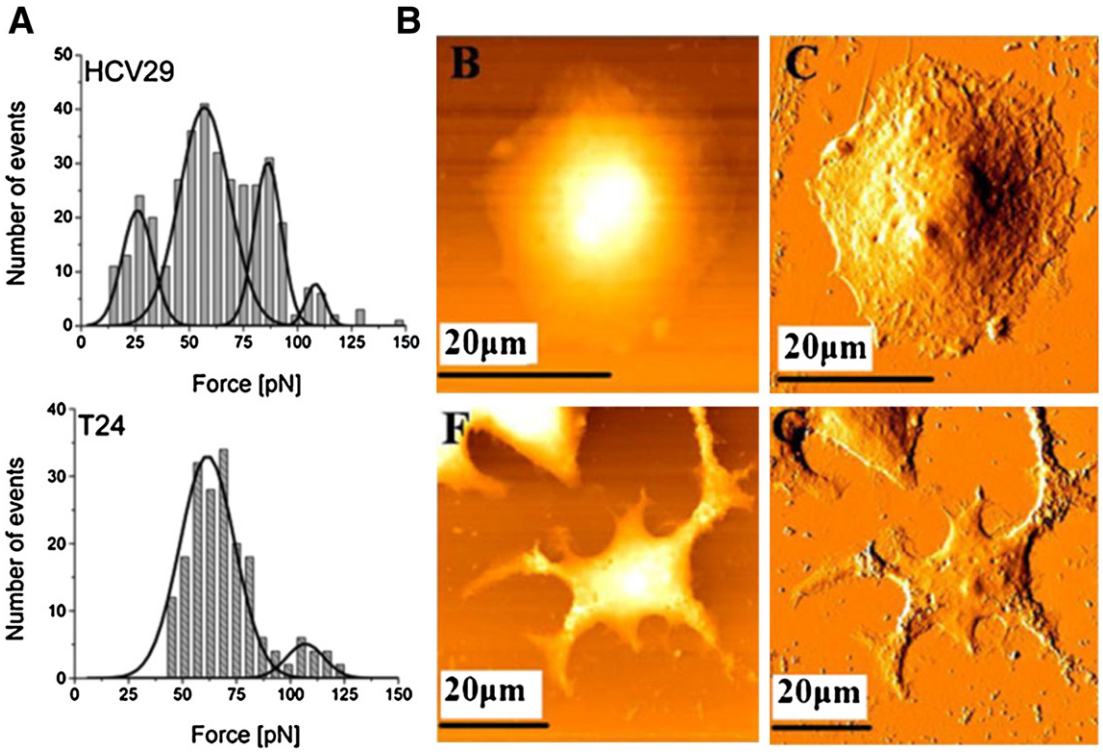


**Fig. 16.** Direct link between cancerous cell elasticity and their interaction with environment. (A) Distribution of the unbinding force for N-cadherin–GC4 complex in non-malignant HCV29 cells and malignant T24 bladder cells. (B) Morphological changes of MCF-7 cells treated without (B, C) or with (F, G) 30 µg/L BMP2 for 24 h. Reprinted with permission from references [173,174].

importance in cancer cell phenotypes [165–167]. Secondly, stiffness has been correlated to the capacity of invasion too as shown in Fig. 16C on ovarian cancer cells [168] or prostate cancer cells [169]. These invasive properties were also determined by the cells ability to adhere differently to the substrate depending on its nature [164,169]. Therefore, it was shown that the rigidity of the substrate influences the invasive cell response as the invasive mechanism implies the degradation of extracellular matrix: cells are then able to sense their environment and choose where to invade [170]. Likewise, during their invasion process, cancer cells can adhere to other cells, and this can be measured by direct cell–cell interactions for example between prostate metastatic cells known to form bone metastases and bone cells [171]. Reeves et al. have demonstrated the ability of prostate cancer cells fixed on the AFM tip to form contact with bone marrow endothelial cells fixed on the substrate [172]. By blocking extracellular part of trans-membrane protein such as integrin and selectin using specific antibodies, they observed a decrease in adhesion events between the two cell types. This result has demonstrated that cell–cell interaction in invasive process is performed through these specific proteins. Following the same idea, N-cadherin levels (calcium adhesion transmembrane proteins, that are characteristic of various cancers and involved in cell adhesive properties) have been measured on cancer and normal bladder cell surface with an antibody fixed on the AFM tip [173]. Results showed an enhanced level of the protein in cancer cells accompanied by higher unbinding forces, meaning that N-cadherin protein is more stable in cancerous cells (Fig. 17A). Furthermore, the presence of cell referenced as good secondary site for metastasis may enhance the invasion capacities of the cancer cells, as shown by Jin et al. on breast cancer cells that change their shape and make specialized migration structures in presence of the bone morphogenetic protein 2 (Fig. 17B) [174].

Cancerous cell changes in gene expression are directly related to the alteration of their function. The data presented above shows that morphological and force spectroscopy investigation help in resolving and understanding cancer cell mechanism and behavior.

*4.2.2. Cancer treatment*

After the determination of cancer cell characteristics and its subsequent link to their behavior, the treatment of patient by eradication of tumor is a second challenge for scientists. We will describe in this section the benefice of AFM for treatment efficiency and mode of action studies.

The first step regarding the efficiency of a therapeutic agent is the targeting of the cancer cells and its delivery. Indeed, most of the current chemical does not specifically affect the cancerous cells and thus need to be addressed. AFM can help in determining this targeting by the study of nanocarriers: nanoparticle interaction with melanoma cells [175] or nanoliposomes containing the well-known cisplatin drug intra cellular delivery in ovarian cancer cells [176]. These nanoliposomes have been shown to be well internalized in the cytoplasm in the size range of 100–300 nm (Fig. 18A). Adhesion specificity discrimination of chemical groups to enhance the targeting of cancerous cell by these

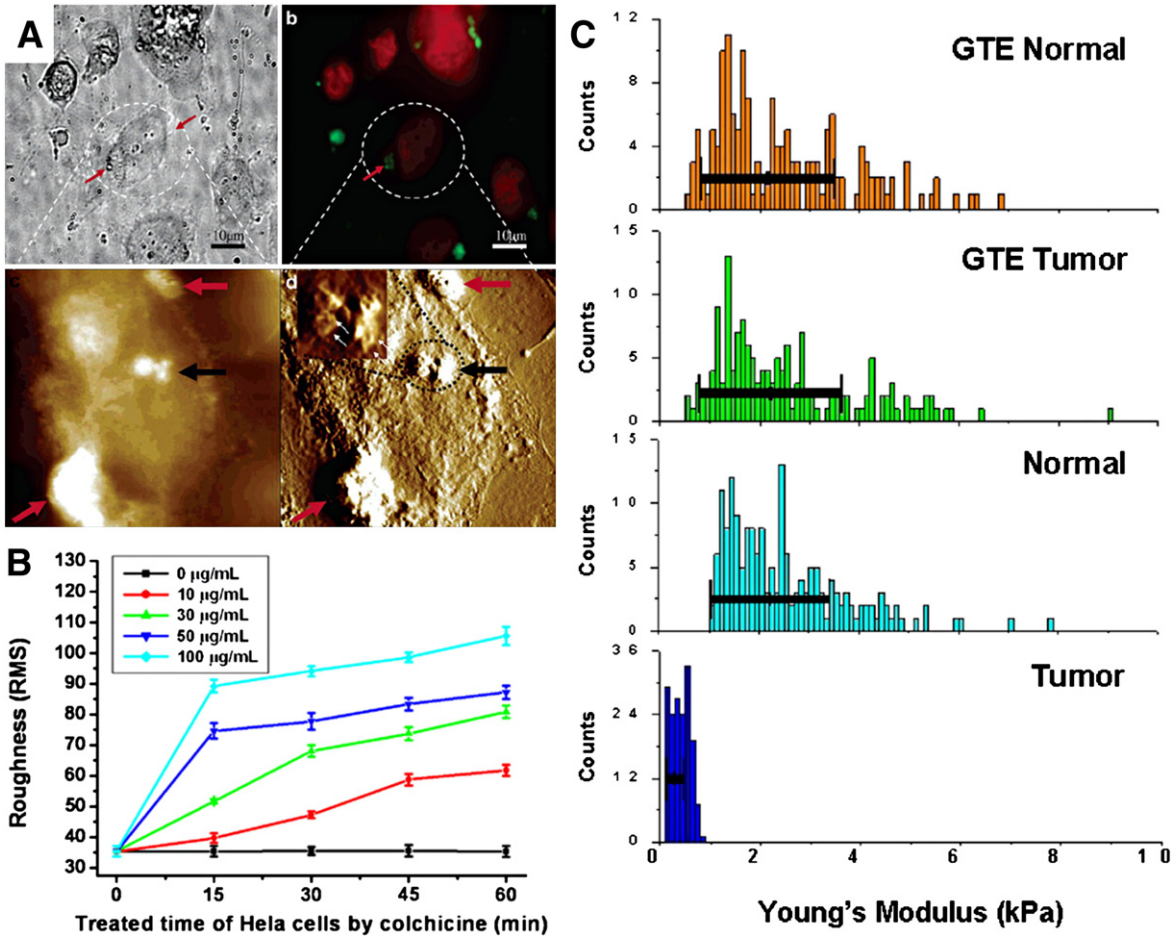


**Fig. 17.** Cancer treatment effects. (A) Internalization of small liposomes containing cisplatin in human ovarian cancer cell line (A2780) after 1 h incubation, top left: contrast phase image, top right: fluorescence images nucleus in red and liposomes in green, bottom left: height image, bottom right: error image. Black arrow: pointing at liposomes on cell surface, red arrows: in the cytoplasm. (B) Root–mean–square roughness variation of cell Hela cell membrane along time depending on colchicine dose. (C) Young's modulus of patient lung metastatic tumor and normal mesothelial cells before (ctrl) and after treatment with Green Tea Extract (GTE) for 24 h. Reprinted with permission from references [176,184,193].

nanocarriers can be measured directly on cells by functionalizing the AFM tip with the chemical itself [177]. The functionalization with these type of carriers can also be achieved through antibodies, for example against MUC-1, a cell surface marker for prostate, breast, and lung cancer [178]. Some antibodies are able, not only to target the cancer cells, but also to provoke apoptosis of the cell. This is the case of antibodies against epidermal growth factor receptor HER2, namely Trastzumab and Pertuzumab, which inhibit the dimerization of HER2 with other epidermal growth factor [179]. This method has also been used to study two cells interaction and helped to solve Zoledronic acid role on prostate cancer that reduced the adhesive interactions between cancer cells and bone marrow endothelial cells [172].

As stated in the previous section, interaction with their environment is of importance for cancerous cells. It is one of the effects of immune system on cancer cells, as shown by Braet et al. that demonstrated that natural killer cells affect the adhesion to substrate properties of colon carcinoma cells [180]. As membrane is the interface with cell's environment, it is one of the key actors that scientist look at when investigating anticancer treatment effects. For example, electropermeabilization treatment effect can be investigated by AFM [181]. Imaging cell surface after treating them with new drugs gives access to the way that drugs affect the cell and can help improving it. Some *ex-vivo* studies performed on lipid bilayers helped in defining the mechanism of action of anti-cancer compounds by imaging breast cancer cell native membrane rafts [182] or Latarcin2a peptide lytic activity on model membranes [183]. Several drug have been studied by imaging membrane on the complete cell: Colchicine on different organs carcinoma cells (dose dependent increase in roughness, Fig. 1) [184], Celecoxib on human colorectal cells [185], Paclitaxel on carcinoma cells [186,187], Alterporiol on breast cancer cells (dose dependent increase in membrane particule size) [188], lithium unexpected effect on carcinoma cells [189] and curcumin anti-cancer effect on liver cells [190]. Membrane is also the site where interactions with the environment occur, and the drug Celastrol affecting the invading capacity of endothelial cell in angiogenesis has been shown to reduce adhesion/affinity to fibronectin protein [191]. Ones must not forget that some drugs are affecting inner cell's components, like the bacterial protein Azurin that stabilize the p53 protein and thus enable apoptosis. The molecular details of this interaction have been assessed by AFM [192].

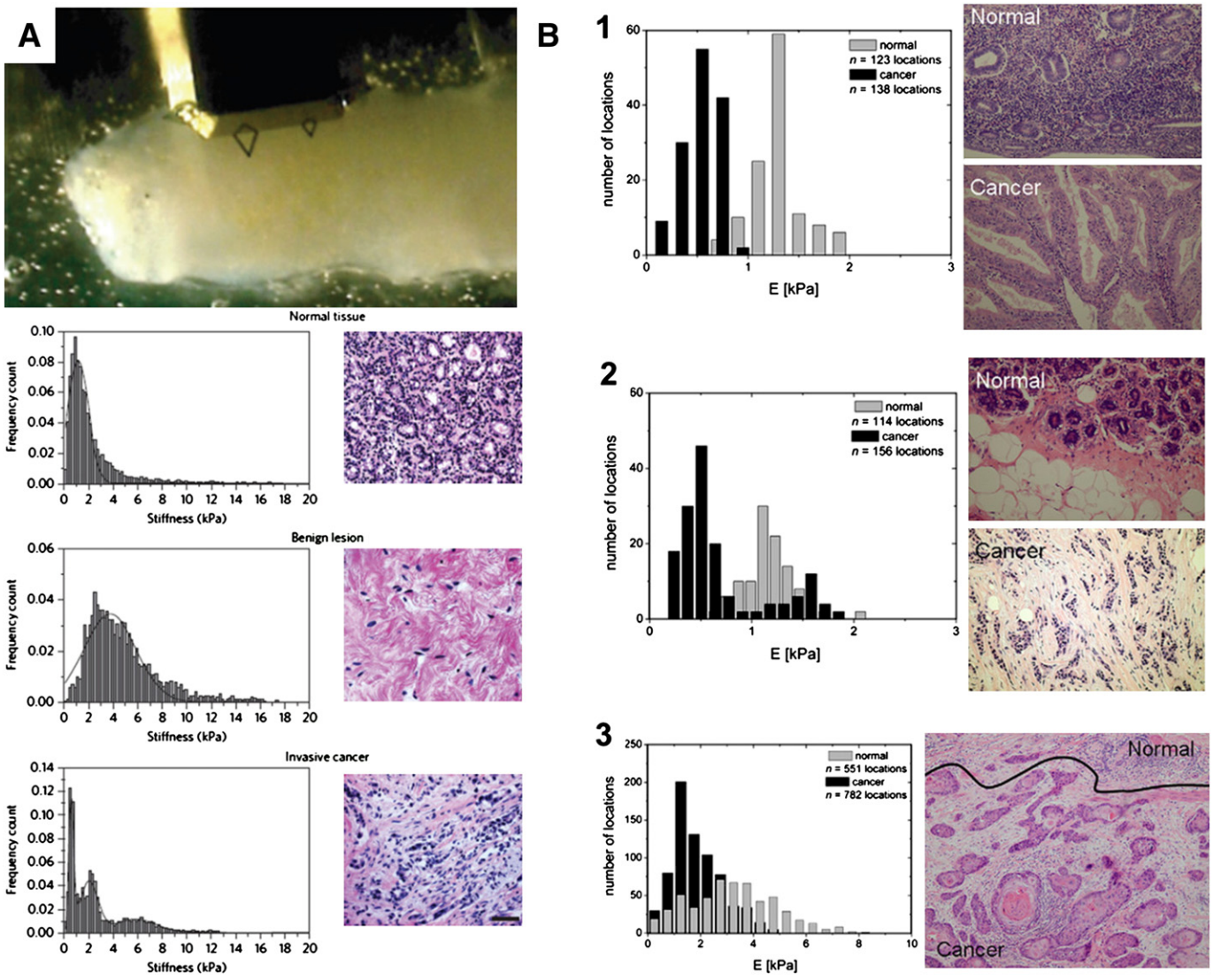


**Fig. 18.** Detection of cancerous cell from patient. (A) Top view of an oriented, immobilized biopsy with the cantilever positioned for AFM measurement. Scale bar, 500 μm. Elasticity of human breast tissue at different step of malignancy. (B) Stiffness distributions in tissues sections accompanied by the corresponding histological staining. (1) Nonneoplastic endometrium (gray columns) and well differentiated endometrioid carcinoma (black columns) of the uterine corpus. (2) Nonneoplastic breast tissue (gray columns) and infiltrating ductal carcinoma (black columns). (3) Vulvar cancer. Black columns denote cancer while gray ones, non-neoplastic parts of the tissue section separated by black line in the histological image. Reprinted with permission from references [198,210].

Of course the elasticity of the cell stays a good parameter to judge on the efficiency of a therapeutic molecule, as for example green tea extract that restores selectively the elasticity of cancerous cells thus confirming its anti-cancer non-destructive activity (Fig. 18C) [193]. However, depending on the effect or the cell, elasticity can show a different tendency. For example, elasticity can reflect the apoptotic effect of a drug, then ones must be looking at a decrease in Young modulus values as it is the case for Paclitaxel [186], and an increase in elasticity can label the resistance of tumor cells to an apoptotic agent like for the TRAIL (TNF-related apoptosis inducing ligand) [194].

*4.2.3. Futures applications*

We have described above the use of AFM for *in vitro* analysis of cancer cells, their properties, and their response to treatment. However the AFM can also be used as a diagnosis tool. *In vitro*, the discrimination between cancerous cells and healthy ones can be achieved by several assays. First, imaging cells can give answers to the question "are these cells cancerous?". The down-regulation of tumor suppressor protein as the matrix associated region protein SMAR1 has been studied by roughness measurement at the surface of cancer cells, and an increase in roughness was shown on cancer cells allowing the differentiation between cancer cell and healthy ones [195]. Secondly, adhesion measurements can inform on the glycosylation state of the cell, directly correlated to their cancer state [196]. As previously mentioned, the elasticity of the cell is a reliable measurement that undoubtedly makes the difference between cancer and non-cancer cells. Cross et al. have been the first to study cells from patients and thus gave the first diagnosis on lung, breast, pancreas cells (Fig. 15C) [158], and later adenocarcinoma [197]. The last improvements are turned to biopsies (Fig. 18) [173,198,210] and allow the correlation between cancer phenotypes and stiffness. These studies open up the use of AFM for bio-medical application of interest for clinical applications, and recent advances in force-curve analysis may help to finely tune these features [199,200].

## 5. Conclusion

Imaging cells at high resolution is of first importance in biology. The atomic force microscope cannot now be ignored because of its advantages. AFM works in liquid, thus potentially on living cells, with a resolution close to the nanometer (depending on the application). But, as exposed in the review, AFM is more than an imaging tool. It is able to measure forces, and gives access to the nanomechanical properties of cells, and/or to the localization of proteins, receptors, at the cell surface. We have reviewed the main domain of application of AFM on living cells: fungal cells, bacteria and mammal cells. However, AFM is also useful to study isolated proteins, DNA, and other biomolecules. We have shown, by reviewing the literature, that AFM experiments give an original vision of cells. It is of first interest to combine AFM analysis with other imaging techniques (MET, SEM, optical microscope, confocal microscope) to get a better understanding of the structure–function relationships. For example, by combining force measurements and transmission electronic microscope, it is possible to make a link between a stiffer structure and the underlying organelles that are present in the cell. The combination with chemical analysis like XPS or SIMS [201] also enlightens the relation between surface structures and chemical composition.

AFM technologies are continuously improving. Among others, we should cite high speed AFM [202,203], high speed force spectroscopies [204], multi-harmonic AFM [205], or the use of AFM as a microelectromechanical system [206]. These developments tend to increase our ability to track fast events occurring at the cell surface and contribute to redefine our understanding of the living cell surface. The next challenge is now to use AFM on living cells, in order to solve a biological problem, and not only to observe biological phenomena. This has been achieved, for example, for the early diagnosis of osteoarthritis [207], the determination of the cataract molecular mechanism [208,209], or for making the difference between normal and cancerous cells [210] (on cell lines, cells coming from patients and biopsis). Classical AFM has been combined with optical, confocal, fluorescence microscopy for several years now [211,212]. The next challenge is to combine these techniques with advanced AFM technologies as it has been recently done for optical microscopy and high speed AFM to observe single membrane proteins on eukaryotic cells [213,214]. The tracking of single molecules by FRET or TIRFF, on living cells, combined with AFM, is also an exciting perspective [215].

In this review we specially addressed the contribution of AFM in pharmacology. We exposed results obtained on fungal cells, bacteria and mammal cells exposed to drugs or encountering diseases. This particular point of view demonstrates that AFM is not providing only fundamental knowledge on cells but is now more and more contributing to studies with medical relevance.

## References


[1] G. Binnig, H. Rohrer, C. Gerber, E. Weibel, Tunneling through a controllable vacuum gap, Appl. Phys. Lett. 40 (1982) 178–180.
[2] G. Binnig, C.F. Quate, Atomic force microscope, Phys. Rev. Lett. 56 (1986) 930–933.
[3] C. Gerber, H.P. Lang, How the doors of the nanoworld were opened, Nat. Nanotechnol. 1 (2006) 3–5.
[4] R.P. Feynman, Feynman's Talk, 1959.
[5] D.M. Eigler, E.K. Schweizer, Positioning single atoms with a scanning tunnelling microscope, Nature 344 (1990) 524–526.
[6] C. Toumey, 35 atoms that changed the nanoworld, Nat. Nanotechnol. 5 (2010) 239–241.
[7] P.K. Hansma, V.B. Elings, O. Marti, C.E. Bracker, Scanning tunneling microscopoy and atomic force microscopy : application to biology and technology, Science 247 (1988) 209–216.
[8] S. Liu, Y. Wang, Application of AFM in microbiology: a review, Scanning 32 (2010) 61–73.
[9] D.P. Allison, N.P. Mortensen, C.J. Sullivan, M.J. Doktycz, Atomic force microscopy of biological samples, Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnol. 2 (2010) 618–634.
[10] G. Binnig, C.F. Quate, C. Gerber, Atomic Force Microscope, Phys. Rev. Lett. 56 (1986) 930–933.
[11] J.D. Ng, Y.G. Kuznetsov, A.J. Malkin, G. Keith, R. Giegé, A. McPherson, Visualization of RNA crystal growth by atomic force microscopy, Nucleic Acids Res. 25 (1997) 2582–2588.
[12] H.G. Hansma, K. Kasuya, E. Oroudjev, Atomic force microscopy imaging and pulling of nucleic acids, Curr. Opin. Struct. Biol. 14 (2004) 380–385.
[13] H. Seelert, A. Poetsch, N.A. Dencher, A. Engel, H. Stahlberg, D.J. Müller, Structural biology: proton-powered turbine of a plant motor, Nature 405 (2000) 418–419.
[14] L.I. Pietrasanta, D. Thrower, W. Hsieh, S. Rao, O. Stemmann, J. Lechner, et al., Probing the *Saccharomyces cerevisiae* centromeric DNA (CEN DNA)-binding factor 3 (CBF3) kinetochore complex by using atomic force microscopy, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 3757–3762.
[15] M. Gaczynska, P.A. Osmulski, Y. Jiang, J.-K. Lee, V. Bermudez, J. Hurwitz, Atomic force microscopic analysis of the binding of the *Schizosaccharomyces pombe* origin recognition complex and the spOrc4 protein with origin DNA, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17952–17957.
[16] Y. Wu, J.S. Siino, T. Sugiyama, S.C. Kowalczykowski, The DNA binding preference of RAD52 and RAD59 proteins implications for RAD52 and RAD59 protein function in homologous recombination, J. Biol. Chem. 281 (2006) 40001–40009.
[17] M. Gad, A. Ikai, Method for immobilizing microbial cells on gel surface for dynamic AFM studies, Biophys. J. 69 (1995) 2226–2233.
[18] R. de Souza Pereira, M.I.N. da Silva, M.A. Cotta, Adhesion forces measured between a calcium blocker drug and its receptor in living cells using atomic force microscope, FEBS Lett. 552 (2003) 155–159.
[19] A.K. Adya, E. Canetta, G.M. Walker, Atomic force microscopic study of the influence of physical stresses on *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, FEMS Yeast Res. 6 (2006) 120–128.
[20] A. Touhami, B. Nysten, Y.F. Dufrêne, Nanoscale mapping of the elasticity of microbial cells by Atomic Force Microscopy, Langmuir 19 (2003) 4539–4543.
[21] E. Dague, R. Bitar, H. Ranchon, F. Durand, H.M. Yken, J.M. François, An atomic force microscopy analysis of yeast mutants defective in cell wall architecture, Yeast 27 (2010) 673–684.
[22] E. Dague, E. Jauvert, L. Laplatine, B. Viallet, C. Thibault, L. Ressier, Assembly of live micro-organisms on microstructured PDMS stamps by convective/capillary deposition for AFM bio-experiments, Nanotechnology 22 (2011) 395102.
[23] E. Dague, D. Alsteens, J.-P. Latgé, Y.F. Dufrêne, High-resolution cell surface dynamics of germinating *Aspergillus fumigatus Conidia*, Biophys. J. 94 (2008) 656–660.
[24] R. De Souza Pereira, N.A. Parizotto, V. Baranauskas, Observation of baker's yeast strains used in biotransformation by atomic force microscopy, Appl. Biochem. Biotechnol. 59 (1996) 135–143.
[25] A. Kriznik, M. Bouillot, J. Coulon, F. Gaboriaud, Morphological specificity of yeast and filamentous *Candida albicans* forms on surface properties, C. R. Biol. 328 (2005) 928–935.

[26] D. Alsteens, V. Dupres, S. Yunus, J.-P. Latgé, J.J. Heinisch, Y.F. Dufrêne, High-resolution imaging of chemical and biological sites on living cells using peak force tapping atomic force microscopy, Langmuir 28 (2012) 16738–16744.

[27] E. Canetta, G.M. Walker, A.K. Adya, Correlating yeast cell stress physiology to changes in the cell surface morphology: atomic force microscopic studies, Sci. World J. 6 (2006) 777–780.

[28] L. Zhao, D. Schaefer, H. Xu, S.J. Modi, W.R. LaCourse, M.R. Marten, Elastic properties of the cell wall of *Aspergillus nidulans* studied with Atomic Force Microscopy, Biotechnol. Prog. 21 (2005) 292–299.

[29] A.E. Pelling, S. Sehati, E.B. Gralla, J.S. Valentine, J.K. Gimzewski, Local nanomechanical motion of the cell wall of *Saccharomyces cerevisiae*, Science 305 (2004) 1147–1150.

[30] A.E. Pelling, S. Sehati, E.B. Gralla, J.K. Gimzewski, Time dependence of the frequency and amplitude of the local nanomechanical motion of yeast, Nanomedicine: Nanotechnology Biol. Med. 1 (2005) 178–183.

[31] W.F. Heinz, J.H. Hoh, Spatially resolved force spectroscopy of biological surfaces using the atomic force microscope, Trends Biotechnol. 17 (1999) 143–150.

[32] R.J. Karreman, E. Dague, F. Gaboriaud, F. Quilès, J.F.L. Duval, G.G. Lindsey, The stress response protein Hsp12p increases the flexibility of the yeast *Saccharomyces cerevisiae* cell wall, Biochimica et Biophysica Acta (BBA)—Proteins and Proteomics 1774 (2007) 131–137.

[33] H. Hertz, Ueber die Berührung fester elastischer Körper, Journal Für Die Reine Und Angewandte Mathematik. 1882 (1881) 156–171.

[34] T. Svaldo-Lanero, S. Krol, R. Magrassi, A. Diaspro, R. Rolandi, A. Gliozzi, et al., Morphology, mechanical properties and viability of encapsulated cells, Ultramicroscopy 107 (2007) 913–921.

[35] P. Chen, H.-H. Liu, R. Cui, Z.-L. Zhang, D.-W. Pang, Z.-X. Xie, et al., Visualized investigation of yeast transformation induced with Li + and polyethylene glycol, Talanta 77 (2008) 262–268.

[36] D. Alsteens, V. Dupres, K. Mc Evoy, L. Wildling, H.J. Gruber, Y.F. Dufrêne, Structure, cell wall elasticity and polysaccharide properties of living yeast cells, as probed by AFM, Nanotechnology 19 (2008) 384005.

[37] P. Hinterdorfer, Y.F. Dufrêne, Detection and localization of single molecular recognition events using atomic force microscopy, Nat. Methods 3 (2006) 347–355.

[38] P. Hinterdorfer, M.F. Garcia-Parajo, Y.F. Dufrêne, Single-molecule imaging of cell surfaces using near-field nanoscopy, Acc. Chem. Res. 45 (2012) 327–336.

[39] Y.F. Dufrêne, P. Hinterdorfer, Recent progress in AFM molecular recognition studies, Pflugers Arch.—Eur. J. Physiol. 456 (2008) 237–245.

[40] M. Gad, Mapping cell wall polysaccharides of living microbial cells using atomic force microscopy, Cell Biol. Int. 21 (1997) 697–706.

[41] A. Beaussart, D. Alsteens, S. El-Kirat-Chatel, P.N. Lipke, S. Kucharíková, P. Van Dijck, et al., Single-molecule imaging and functional analysis of als adhesins and mannans during *Candida albicans* morphogenesis, ACS Nano 6 (2012) 10950–10964.

[42] D.E. Levin, Cell wall integrity signaling in *Saccharomyces cerevisiae*, Microbiol. Mol. Biol. Rev. 69 (2005) 262–291.

[43] J.J. Heinisch, V. Dupres, S. Wilk, A. Jendretzki, Y.F. Dufrêne, Single-molecule atomic force microscopy reveals clustering of the yeast plasma-membrane sensor Wsc1, PLoS One 5 (2010).

[44] V. Dupres, Y.F. Dufrêne, J.J. Heinisch, Measuring cell wall thickness in living yeast cells using single molecular rulers, ACS Nano 4 (2010) 5498–5504.

[45] D. Alsteens, M.C. Garcia, P.N. Lipke, Y.F. Dufrêne, Force-induced formation and propagation of adhesion nanodomains in living fungal cells, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 20744–20749.

[46] C. Mora, D.P. Tittensor, S. Adl, A.G.B. Simpson, B. Worm, How many species are there on earth and in the ocean? PLoS Biol. 9 (2011) e1001127.

[47] G.D. Brown, D.W. Denning, S.M. Levitz, Tackling human fungal infections, Science 336 (2012) 647.

[48] F.L. Mayer, D. Wilson, B. Hube, *Candida albicans* pathogenicity mechanisms, Virulence 4 (2013) 119–128.

[49] K.S. Kim, Y.-S. Kim, I. Han, M.-H. Kim, M.H. Jung, H.-K. Park, Quantitative and qualitative analyses of the cell death process in *Candida albicans* treated by antifungal agents, PLoS ONE 6 (2011) e28176.

[50] Y.-S. Kim, K.S. Kim, I. Han, M.-H. Kim, M.H. Jung, H.-K. Park, Quantitative and qualitative analysis of the antifungal activity of allicin alone and in combination with antifungal drugs, PLoS One 7 (2012).

[51] M. Götzinger, B. Weigl, W. Peukert, K. Sommer, Effect of roughness on particle adhesion in aqueous solutions: a study of *Saccharomyces cerevisiae* and a silica particle, Colloids Surf. B: Biointerfaces 55 (2007) 44–50.

[52] E.S. Ovchinnikova, B.P. Krom, H.J. Busscher, H.C. van der Mei, Evaluation of adhesion forces of *Staphylococcus aureus* along the length of *Candida albicans hyphae*, BMC Microbiol. 12 (2012) 281.

[53] A. Beaussart, P. Herman, S. El-Kirat-Chatel, P.N. Lipke, S. Kucharíková, P. Van Dijck, et al., Single-cell force spectroscopy of the medically important *Staphylococcus epidermidis–Candida albicans* interaction, Nanoscale (2013) 10894–10900.

[54] S. El-Kirat-Chatel, Y.F. Dufrêne, Nanoscale imaging of the *Candida*–Macrophage interaction using correlated fluorescence-atomic force microscopy, ACS Nano 6 (2012) 10792–10799.

[55] F. Comitini, I. Mannazzu, M. Ciani, Tetrapisispora phaffii killer toxin is a highly specific β-glucanase that disrupts the integrity of the yeast cell wall, Microb. Cell Factories 8 (2009) 55.

[56] A.K. Tyagi, A. Malik, In situ SEM, TEM and AFM studies of the antimicrobial activity of lemon grass oil in liquid and vapour phase against *Candida albicans*, Micron 41 (2010) 797–805.

[57] I.B. Beech, J.R. Smith, A.A. Steele, I. Penegar, S.A. Campbell, The use of atomic force microscopy for studying interactions of bacterial biofilms with surfaces, Colloids Surf. B: Biointerfaces 23 (2002) 231–247.

[58] A. Touhami, M.H. Jericho, J.M. Boyd, T.J. Beveridge, Nanoscale characterization and determination of adhesion forces of *Pseudomonas aeruginosa* pili by using atomic force microscopy, J. Bacteriol. 188 (2006) 370–377.

[59] J. Rheinlaender, A. Gräbner, L. Ott, A. Burkovski, T.E. Schäffer, Contour and persistence length of *Corynebacterium diphtheriae* pili by atomic force microscopy, Eur. Biophys. J. 41 (2012) 561–570.

[60] P. Tripathi, V. Dupres, A. Beaussart, S. Lebeer, I.J.J. Claes, J. Vanderleyden, et al., Deciphering the nanometer-scale organization and assembly of *Lactobacillus rhamnosus* GG pili using atomic force microscopy, Langmuir 28 (2012) 2211–2216.

[61] A. Gillis, V. Dupres, J. Mahillon, Y.F. Dufrêne, Atomic force microscopy: a powerful tool for studying bacterial swarming motility, Micron 43 (2012) 1304–1311.

[62] A. Gillis, V. Dupres, G. Delestrait, J. Mahillon, Y.F. Dufrêne, Nanoscale imaging of *Bacillus thuringiensis* flagella using atomic force microscopy, Nanoscale 4 (2012) 1585–1591.

[63] C. Díaz, P.L. Schilardi, R.C. Salvarezza, M. Fernández Lorenzo de Mele, Have flagella a preferred orientation during early stages of biofilm formation?: AFM study using patterned substrates, Colloids Surf. B: Biointerfaces 82 (2011) 536–542.

[64] K.-C. Chang, S.-J. Cheng, Y.-C. Chen, H.-R. Huang, J.-W. Liou, Nanoscopic analysis on pH induced morphological changes of flagella in *Escherichia coli*, J. Microbiol. Immunol. Infect. (2012), in press.

[65] H.-N. Su, Z.-H. Chen, S.-B. Liu, L.-P. Qiao, X.-L. Chen, H.-L. He, et al., Characterization of bacterial polysaccharide capsules and detection in the presence of deliquescent water by atomic force microscopy, Appl. Environ. Microbiol. 78 (2012) 3476–3479.

[66] O. Stukalov, A. Korenevsky, T.J. Beveridge, J.R. Dutcher, Use of atomic force microscopy and transmission electron microscopy for correlative studies of bacterial capsules, Appl. Environ. Microbiol. 74 (2008) 5457–5465.

[67] Z. Suo, X. Yang, R. Avci, L. Kellerman, D.W. Pascual, M. Fries, et al., HEPES-stabilized encapsulation of *Salmonella typhimurium*, Langmuir 23 (2007) 1365–1374.

[68] F.M. Coldren, E.L. Palavecino, N.H. Levi-Polyachenko, W.D. Wagner, T.L. Smith, B.P. Smith, et al., Encapsulated *Staphylococcus aureus* strains vary in adhesiveness assessed by atomic force microscopy, J. Biomed. Mater. Res. A 89A (2009) 402–410.

[69] V. Dupres, D. Alsteens, K. Pauwels, Y.F. Dufrêne, In vivo imaging of S-layer nanoarrays on *Corynebacterium glutamicum*, Langmuir 25 (2009) 9653–9655.

[70] J. Tang, A. Ebner, B. Kraxberger, M. Leitner, A. Hykollari, C. Kepplinger, et al., Detection of metal binding sites on functional S-layer nanoarrays using single molecule force spectroscopy, J. Struct. Biol. 168 (2009) 217–222.

[71] C. Horejs, R. Ristl, R. Tscheliessnig, U.B. Sleytr, D. Pum, Single-molecule force spectroscopy reveals the individual mechanical unfolding pathways of a surface layer protein, J. Biol. Chem. 286 (2011) 27416–27424.

[72] M. Saif Zaman, A. Goyal, G. Prakash Dubey, P.K. Gupta, H. Chandra, T.K. Das, et al., Imaging and analysis of *Bacillus anthracis* spore germination, Microsc. Res. Tech. 66 (2005) 307–311.

[73] M. Plomp, T.J. Leighton, K.E. Wheeler, M.E. Pitesky, A.J. Malkin, *Bacillus atrophaeus* outer spore coat assembly and ultrastructure, Langmuir 21 (2005) 10710–10716.

[74] P.A. Pinzón-Arango, G. Scholl, R. Nagarajan, C.M. Mello, T.A. Camesano, Atomic force microscopy study of germination and killing of Bacillusatrophaeus spores, J. Mol. Recognit. 22 (2009) 373–379.

[75] M. Plomp, T.J. Leighton, K.E. Wheeler, H.D. Hill, A.J. Malkin, In vitro high-resolution structural dynamics of single germinating bacterial spores, PNAS 104 (2007) 9644–9649.

[76] G. Francius, P. Polyakov, J. Merlin, Y. Abe, J.-M. Ghigo, C. Merlin, et al., Bacterial surface appendages strongly impact nanomechanical and electrokinetic properties of *Escherichia coli* cells subjected to osmotic stress, PLoS ONE 6 (2011) e20066.

[77] P. Schaer-Zammaretti, J. Ubbink, Imaging of lactic acid bacteria with AFM—elasticity and adhesion maps and their relationship to biological and structural data, Ultramicroscopy 97 (2003) 199–208.

[78] F. Gaboriaud, S. Bailet, E. Dague, F. Jorand, Surface structure and nanomechanical properties of *Shewanella putrefaciens* bacteria at two pH values (4 and 10) determined by atomic force microscopy, J. Bacteriol. 187 (2005) 3864–3868.

[79] A. Cerf, J.-C. Cau, C. Vieu, E. Dague, Nanomechanical properties of dead or alive single-patterned bacteria, Langmuir 25 (2009) 5731–5736.

[80] C. Roduit, B. Saha, L. Alonso-Sarduy, A. Volterra, G. Dietler, S. Kasas, OpenFovea: open-source AFM data processing software, Nat. Methods 9 (2012) 774–775.

[81] G. Longo, L.M. Rio, C. Roduit, A. Trampuz, A. Bizzini, G. Dietler, et al., Force volume and stiffness tomography investigation on the dynamics of stiff material under bacterial membranes, J. Mol. Recognit. 25 (2012) 278–284.

[82] E.V. Dubrovin, A.G. Voloshin, S.V. Kraevsky, T.E. Ignatyuk, S.S. Abramchuk, I.V. Yaminsky, et al., Atomic force microscopy investigation of phage infection of bacteria, Langmuir 24 (2008) 13068–13074.

[83] E.V. Dubrovin, A.V. Popova, S.V. Kraevskiy, S.G. Ignatov, T.E. Ignatyuk, I.V. Yaminsky, et al., Atomic force microscopy analysis of the *Acinetobacter baumannii* bacteriophage AP22 lytic cycle, PLoS ONE 7 (2012) e47348.

[84] C. Verbelen, Y.F. Dufrêne, Direct measurement of Mycobacterium–fibronectin interactions, Integr. Biol. 1 (2009) 296–300.

[85] E. Dague, D.T.L. Le, S. Zanna, P. Marcus, P. Loubière, M. Mercier-Bonin, Probing in vitro interactions between *Lactococcus lactis* and mucins using AFM, Langmuir 26 (2010) 11010–11017.

[86] D.T.L. Le, Y. Guérardel, P. Loubière, M. Mercier-Bonin, E. Dague, Measuring kinetic dissociation/association constants between *Lactococcus lactis* bacteria and mucins using living cell probes, Biophys. J. 101 (2011) 2843–2853.

[87] M. Firtel, G. Henderson, I. Sokolov, Nanosurgery: observation of peptidoglycan strands in *Lactobacillus helveticus* cell walls, Ultramicroscopy 101 (2004) 105–109.

[88] G. Andre, S. Kulakauskas, M.-P. Chapot-Chartier, B. Navet, M. Deghorain, E. Bernard, et al., Imaging the nanoscale organization of peptidoglycan in living *Lactococcus lactis* cells, Nat. Commun. 1 (2010) 27.

[89] R. Wheeler, S. Mesnage, I.G. Boneca, J.K. Hobbs, S.J. Foster, Super-resolution microscopy reveals cell wall dynamics and peptidoglycan architecture in ovococcal bacteria, Mol. Microbiol. 82 (2011) 1096–1109.
[90] E.J. Hayhurst, L. Kailas, J.K. Hobbs, S.J. Foster, Cell wall peptidoglycan architecture in *Bacillus subtilis*, PNAS 105 (2008) 14603–14608.
[91] G. Francius, S. Lebeer, D. Alsteens, L. Wildling, H.J. Gruber, P. Hols, et al., Detection, localization, and conformational analysis of single polysaccharide molecules on live bacteria, ACS Nano 2 (2008) 1921–1929.
[92] J. Strauss, N.A. Burnham, T.A. Camesano, Atomic force microscopy study of the role of LPS O-antigen on adhesion of *E. coli*, J. Mol. Recognit. 22 (2009) 347–355.
[93] J. Tang, D. Krajcikova, R. Zhu, A. Ebner, S. Cutting, H.J. Gruber, et al., Atomic force microscopy imaging and single molecule recognition force spectroscopy of coat proteins on the surface of *Bacillus subtilis* spore, J. Mol. Recognit. 20 (2007) 483–489.
[94] M. Plomp, A.J. Malkin, Mapping of proteomic composition on the surfaces of Bacillus spores by atomic force microscopy-based immunolabeling, Langmuir 25 (2009) 403–409.
[95] V. Dupres, F.D. Menozzi, C. Locht, B.H. Clare, N.L. Abbott, S. Cuenot, et al., Nanoscale mapping and functional analysis of individual adhesins on living bacteria, Nat. Methods 2 (2005) 515–520.
[96] M. Grare, De la génèse d'une nouvelle classe d'antibactériens à base de polyphénols cycliques de type calixarène. Etudes moléculaire(s), cellulaire(s), et structurale(s) en vue de l'identification des cibles d'action: le cas du para-guanidinoéthylcalix [4]arène, Université Henri Poincaré, Nancy-I, 2009.
[97] C. Formosa, M. Grare, R.E. Duval, E. Dague, Nanoscale effects of antibiotics on *P. aeruginosa*, Nanomed. Nanotechnol. Biol. Med. 8 (2012) 12–16.
[98] P.C. Braga, D. Ricci, Atomic force microscopy: application to investigation of *Escherichia coli* morphology before and after exposure to cefodizime, Antimicrob. Agents Chemother. 42 (1998) 18–22.
[99] M. Ouberai, F. El Garch, A. Bussiere, M. Riou, D. Alsteens, L. Lins, et al., The *Pseudomonas aeruginosa* membranes: a target for a new amphiphilic aminoglycoside derivative? Biochim. Biophys. Acta Biomembr. 1808 (2011) 1716–1727.
[100] M.T. Montero, M. Pijoan, S. Merino-Montero, T. Vinuesa, J. Hernández-Borrell, Interfacial membrane effects of fluoroquinolones as revealed by a combination of fluorescence binding experiments and atomic force microscopy observations, Langmuir 22 (2006) 7574–7578.
[101] G. Francius, O. Domenech, M.P. Mingeot-Leclercq, Y.F. Dufrêne, Direct observation of *Staphylococcus aureus* cell wall digestion by lysostaphin, J. Bacteriol. 190 (2008) 7904–7909.
[102] Y. Gilbert, M. Deghorain, L. Wang, B. Xu, P.D. Pollheimer, H.J. Gruber, et al., Single-molecule force spectroscopy and imaging of the vancomycin/d-Ala-d-Ala interaction, Nano Lett. 7 (2007) 796–801.
[103] Y. Wu, A. Zhou, In situ, real-time tracking of cell wall topography and nanomechanics of antimycobacterial drugs treated Mycobacterium JLS using atomic force microscopy, Chem. Commun. (2009) 7021–7023.
[104] C. Verbelen, V. Dupres, F.D. Menozzi, D. Raze, A.R. Baulard, P. Hols, et al., Ethambutol-induced alterations in *Mycobacterium bovis* BCG imaged by atomic force microscopy, FEMS Microbiol. Lett. 264 (2006) 192–197.
[105] R.L. Soon, R.L. Nation, M. Harper, B. Adler, J.D. Boyce, C.-H. Tan, et al., Effect of colistin exposure and growth phase on the surface properties of live *Acinetobacter baumannii* cells examined by atomic force microscopy, Int. J. Antimicrob. Agents 38 (2011) 493–501.
[106] R.L. Soon, R.L. Nation, P.G. Hartley, I. Larson, J. Li, Atomic force microscopy investigation of the morphology and topography of colistin-heteroresistant *Acinetobacter baumannii* strains as a function of growth phase and in response to colistin treatment, Antimicrob. Agents Chemother. 53 (2009) 4979–4986.
[107] N.P. Mortensen, J.D. Fowlkes, C.J. Sullivan, D.P. Allison, N.B. Larsen, S. Molin, et al., Effects of colistin on surface ultrastructure and nanomechanics of *Pseudomonas aeruginosa* cells, Langmuir 25 (2009) 3728–3733.
[108] A. da S. Junior, O. Teschke, Dynamics of the antimicrobial peptide PGLa action on *Escherichia coli* monitored by atomic force microscopy, World J. Microbiol. Biotechnol. 21 (2005) 1103–1110.
[109] M. Meincken, D.L. Holroyd, M. Rautenbach, Atomic force microscopy study of the effect of antimicrobial peptides on the cell envelope of *Escherichia coli*, Antimicrob. Agents Chemother. 49 (2005) 4085–4092.
[110] A. Li, P.Y. Lee, B. Ho, J.L. Ding, C.T. Lim, Atomic force microscopy study of the antimicrobial action of Sushi peptides on Gram negative bacteria, Biochim. Biophys. Acta Biomembr. 1768 (2007) 411–418.
[111] G. Francius, S. Dufour, M. Deleu, M. Paquot, M.-P. Mingeot-Leclercq, Y.F. Dufrêne, Nanoscale membrane activity of surfactins: influence of geometry, charge and hydrophobicity, Biochim. Biophys. Acta Biomembr. 1778 (2008) 2058–2068.
[112] M. Arseneault, S. Bédard, M. Boulet-Audet, M. Pézolet, Study of the interaction of lactoferricin B with phospholipid monolayers and bilayers, Langmuir 26 (2010) 3468–3478.
[113] C.Y. Flores, C. Diaz, A. Rubert, G.A. Benítez, M.S. Moreno, M.A. Fernández Lorenzo de Mele, et al., Spontaneous adsorption of silver nanoparticles on Ti/TiO2 surfaces. Antibacterial effect on *Pseudomonas aeruginosa*, J. Colloid Interface Sci. 350 (2010) 402–408.
[114] V. Gopinath, D. MubarakAli, S. Priyadarshini, N.M. Priyadharsshini, N. Thajuddin, P. Velusamy, Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach, Colloids Surf. B: Biointerfaces 96 (2012) 69–74.
[115] A.K. Suresh, D.A. Pelletier, W. Wang, J.-W. Moon, B. Gu, N.P. Mortensen, et al., Silver nanocrystallites: biofabrication using *Shewanella oneidensis*, and an evaluation of their comparative toxicity on Gram-negative and Gram-positive bacteria, Environ. Sci. Technol. 44 (2010) 5210–5215.
[116] A. Pramanik, D. Laha, D. Bhattacharya, P. Pramanik, P. Karmakar, A novel study of antibacterial activity of copper iodide nanoparticle mediated by DNA and membrane damage, Colloids Surf. B: Biointerfaces 96 (2012) 50–55.
[117] M. Grare, M. Mourer, S. Fontanay, J.-B. Regnouf-de-Vains, C. Finance, R.E. Duval, In vitro activity of para-guanidinoethylcalix[4]arene against susceptible and antibiotic-resistant Gram-negative and Gram-positive bacteria, J. Antimicrob. Chemother. 60 (2007) 575–581.
[118] C. Formosa, M. Grare, E. Jauvert, A. Coutable, J.B. Regnouf-de-Vains, M. Mourer, et al., Nanoscale analysis of the effects of antibiotics and CX1 on a *Pseudomonas aeruginosa* multidrug-resistant strain, Sci. Rep. 2 (2012).
[119] M. Mourer, R.E. Duval, C. Finance, J.-B. Regnouf-de-Vains, Functional organisation and gain of activity: the case of the antibacterial tetra-para-guanidinoethyl-calix [4]arene, Bioorg. Med. Chem. Lett. 16 (2006) 2960–2963.
[120] M. Mourer, H. Massimba Dibama, P. Constant, M. Daffé, J.-B. Regnouf-de-Vains, Anti-mycobacterial activities of some cationic and anionic calix[4]arene derivatives, Bioorg. Med. Chem. 20 (2012) 2035–2041.
[121] M.B. Patel, N.R. Modi, J.P. Raval, S.K. Menon, Calix[4]arene based 1,3,4-oxadiazole and thiadiazole derivatives: design, synthesis, and biological evaluation, Org. Biomol. Chem. 10 (2012) 1785–1794.
[122] S. Liu, A.K. Ng, R. Xu, J. Wei, C.M. Tan, Y. Yang, et al., Antibacterial action of dispersed single-walled carbon nanotubes on *Escherichia coli* and *Bacillus subtilis* investigated by atomic force microscopy, Nanoscale 2 (2010) 2744–2750.
[123] P. Eaton, J.C. Fernandes, E. Pereira, M.E. Pintado, F. Xavier Malcata, Atomic force microscopy study of the antibacterial effects of chitosans on *Escherichia coli* and *Staphylococcus aureus*, Ultramicroscopy 108 (2008) 1128–1134.
[124] J.C. Fernandes, P. Eaton, A.M. Gomes, M.E. Pintado, F. Xavier Malcata, Study of the antibacterial effects of chitosans on *Bacillus cereus* (and its spores) by atomic force microscopy imaging and nanoindentation, Ultramicroscopy 109 (2009) 854–860.
[125] J.A. Younes, H.C. van der Mei, E. van den Heuvel, H.J. Busscher, G. Reid, Adhesion forces and coaggregation between vaginal staphylococci and lactobacilli, PLoS ONE 7 (2012) e36917.
[126] A. Beaussart, S. El-Kirat-Chatel, P. Herman, D. Alsteens, J. Mahillon, P. Hols, et al., Single-cell force spectroscopy of probiotic bacteria, Biophys. J. 104 (2013) 1886–1892.
[127] T. Kobiela, K. Lelen-Kaminska, M. Stepulak, M. Lekka, M. Malejczyk, J. Arct, et al., The influence of surfactants and hydrolyzed proteins on keratinocytes viability and elasticity, Skin Res. Technol. 19 (2013) e200–e208.
[128] J. Helenius, C.-P. Heisenberg, H.E. Gaub, D.J. Muller, Single-cell force spectroscopy, J. Cell Sci. 121 (2008) 1785–1791.
[129] X. Zhang, E.P. Wojcikiewicz, V.T. Moy, Dynamic adhesion of T lymphocytes to endothelial cells revealed by atomic force microscopy, Exp. Biol. Med. (Maywood) 231 (2006) 1306–1312.
[130] G. Pfister, C.M. Stroh, H. Perschinka, M. Kind, M. Knoflach, P. Hinterdorfer, et al., Detection of HSP60 on the membrane surface of stressed human endothelial cells by atomic force and confocal microscopy, J. Cell Sci. 118 (2005) 1587–1594.
[131] M. Beckmann, H.A. Kolb, F. Lang, Atomic force microscopy of peritoneal macrophages after particle phagocytosis, J. Membr. Biol. 140 (1994) 197–204.
[132] M. Fritz, M. Radmacher, H.E. Gaub, Granula motion and membrane spreading during activation of human platelets imaged by atomic force microscopy, Biophys. J. 66 (1994) 1328–1334.
[133] S.-W. Han, S. Mieda, C. Nakamura, T. Kihara, N. Nakamura, J. Miyake, Successive detection of insulin-like growth factor-II bound to receptors on a living cell surface using an AFM, J. Mol. Recognit. 24 (2011) 17–22.
[134] D. Qiu, J. Xiang, Z. Li, A. Krishnamoorthy, L. Chen, R. Wang, Profiling TRA-1–81 antigen distribution on a human embryonic stem cell, Biochem. Biophys. Res. Commun. 369 (2008) 735–740.
[135] Y. Sugawara, R. Ando, H. Kamioka, Y. Ishihara, S.A. Murshid, K. Hashimoto, et al., The alteration of a mechanical property of bone cells during the process of changing from osteoblasts to osteocytes, Bone 43 (2008) 19–24.
[136] Domke, Dannöhl, Parak, Müller, Aicher, Radmacher, Substrate dependent differences in morphology and elasticity of living osteoblasts investigated by atomic force microscopy, Colloids Surf. B: Biointerfaces 19 (2000) 367–379.
[137] X. Cai, S. Gao, J. Cai, Y. Wu, H. Deng, Artesunate induced morphological and mechanical changes of Jurkat cell studied by AFM, Scanning 31 (2009) 83–89.
[138] U. Hillebrand, M. Hausberg, D. Lang, C. Stock, C. Riethmüller, C. Callies, et al., How steroid hormones act on the endothelium—insights by atomic force microscopy, Pflugers Arch. 456 (2008) 51–60.
[139] A. Yersin, H. Hirling, S. Kasas, C. Roduit, K. Kulangara, G. Dietler, et al., Elastic properties of the cell surface and trafficking of single AMPA receptors in living hippocampal neurons, Biophys. J. 92 (2007) 4482–4489.
[140] A. Zuk, M. Targosz-Korecka, M. Szymonski, Effect of selected drugs used in asthma treatment on morphology and elastic properties of red blood cells, Int. J. Nanomedicine 6 (2011) 249–257.
[141] Y. Li, J. Zhang, B. Zhang, Atomic force microscopy study on chlorpromazine-induced morphological changes of living HeLa cells In Vitro, Scanning 31 (2009) 259–265.
[142] M. Lasalvia, G. Perna, E. Mezzenga, E. Migliorini, M. Lazzarino, N. L'abbate, et al., Atomic force microscopy investigation of morphological changes in living keratinocytes treated with HgCl(2) at not cytotoxic doses, J. Microsc. 243 (2011) 40–46.
[143] R. Afrin, U.S. Zohora, H. Uehara, T. Watanabe-Nakayama, A. Ikai, Atomic force microscopy for cellular level manipulation: imaging intracellular structures and DNA delivery through a membrane hole, J. Mol. Recognit. 22 (2009) 363–372.

[144] Y. Liu, J. Feng, L. Shi, R. Niu, Q. Sun, H. Liu, et al., In situ mechanical analysis of cardiomyocytes at nano scales, Nanoscale 4 (2012) 99–102.
[145] J.F. Saenz Cogollo, M. Tedesco, S. Martinoia, R. Raiteri, A new integrated system combining atomic force microscopy and micro-electrode array for measuring the mechanical properties of living cardiac myocytes, Biomed. Microdevices 13 (2011) 613–621.
[146] W. Hiesinger, M.J. Brukman, R.C. McCormick, J.R. Fitzpatrick III, J.R. Frederick, E.C. Yang, et al., Myocardial tissue elastic properties determined by atomic force microscopy after stromal cell-derived factor 1α angiogenic therapy for acute myocardial infarction in a murine model, J. Thorac. Cardiovasc. Surg. 143 (2012) 962–966.
[147] G. Genet, C. Guilbeau-Frugier, B. Honton, E. Dague, M.D. Schneider, C. Coatrieux, et al., Ephrin-B1 is a novel specific component of the lateral membrane of the cardiomyocyte and is essential for the stability of cardiac tissue architecture cohesion, Circ. Res. 110 (2012) 688–700.
[148] G.-J. Lee, S.-J. Chae, J.H. Jeong, S.-R. Lee, S.-J. Ha, Y.K. Pak, et al., Characterization of mitochondria isolated from normal and ischemic hearts in rats utilizing atomic force microscopy, Micron 42 (2011) 299–304.
[149] J. Fels, P. Jeggle, K. Kusche-Vihrog, H. Oberleithner, Cortical actin nanodynamics determines nitric oxide release in vascular endothelium, PLoS ONE 7 (2012) e41520.
[150] H. Miyazaki, K. Hayashi, Atomic force microscopic measurement of the mechanical properties of intact endothelial cells in fresh arteries, Med. Biol. Eng. Comput. 37 (1999) 530–536.
[151] J.A. Chouinard, G. Grenier, A. Khalil, P. Vermette, Oxidized-LDL induce morphological changes and increase stiffness of endothelial cells, Exp. Cell Res. 314 (2008) 3007–3016.
[152] P. Eaton, V. Zuzarte-Luis, M.M. Mota, N.C. Santos, M. Prudêncio, Infection by Plasmodium changes shape and stiffness of hepatic cells, Nanomedicine 8 (2012) 17–19.
[153] M. Lekka, P. Laidler, D. Gil, J. Lekki, Z. Stachura, A.Z. Hrynkiewicz, Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy, Eur. Biophys. J. 28 (1999) 312–316.
[154] E.C. Faria, N. Ma, E. Gazi, P. Gardner, M. Brown, N.W. Clarke, et al., Measurement of elastic properties of prostate cancer cells using AFM, Analyst 133 (2008) 1498–1500.
[155] Q.S. Li, G.Y.H. Lee, C.N. Ong, C.T. Lim, AFM indentation study of breast cancer cells, Biochem. Biophys. Res. Commun. 374 (2008) 609–613.
[156] S. Iyer, R.M. Gaikwad, V. Subba-Rao, C.D. Woodworth, I. Sokolov, Atomic force microscopy detects differences in the surface brush of normal and cancerous cells, Nat. Nanotechnol. 4 (2009) 389–393.
[157] M.J. Rosenbluth, W.A. Lam, D.A. Fletcher, Force microscopy of nonadherent cells: a comparison of leukemia cell deformability, Biophys. J. 90 (2006) 2994–3003.
[158] S.E. Cross, Y.-S. Jin, J. Rao, J.K. Gimzewski, Nanomechanical analysis of cells from cancer patients, Nat. Nanotechnol. 2 (2007) 780–783.
[159] M. Lekka, P. Laidler, J. Ignacak, M. Łabedz, J. Lekki, H. Struszczyk, et al., The effect of chitosan on stiffness and glycolytic activity of human bladder cells, Biochim. Biophys. Acta 1540 (2001) 127–136.
[160] A. Mescola, S. Vella, M. Scotto, P. Gavazzo, C. Canale, A. Diaspro, et al., Probing cytoskeleton organisation of neuroblastoma cells with single-cell force spectroscopy, J. Mol. Recognit. 25 (2012) 270–277.
[161] T. Watanabe, H. Kuramochi, A. Takahashi, K. Imai, N. Katsuta, T. Nakayama, et al., Higher cell stiffness indicating lower metastatic potential in B16 melanoma cell variants and in (−)-epigallocatechin gallate-treated cells, J. Cancer Res. Clin. Oncol. (2012) 859–866.
[162] A. Fuhrmann, J.R. Staunton, V. Nandakumar, N. Banyai, P.C.W. Davies, R. Ros, AFM stiffness nanotomography of normal, metaplastic and dysplastic human esophageal cells, Phys. Biol. 8 (2011) 015007.
[163] S. Leporatti, D. Vergara, A. Zacheo, V. Vergaro, G. Maruccio, R. Cingolani, et al., Cytomechanical and topological investigation of MCF-7 cells by scanning force microscopy, Nanotechnology 20 (2009) 055103.
[164] D. Docheva, D. Padula, M. Schieker, H. Clausen-Schaumann, Effect of collagen I and fibronectin on the adhesion, elasticity and cytoskeletal organization of prostate cancer cells, Biochem. Biophys. Res. Commun. 402 (2010) 361–366.
[165] J. Alcaraz, R. Xu, H. Mori, C.M. Nelson, R. Mroue, V.A. Spencer, et al., Laminin and biomimetic extracellular elasticity enhance functional differentiation in mammary epithelia, EMBO J. 27 (2008) 2829–2838.
[166] G. Zhao, J. Cui, Q. Qin, J. Zhang, L. Liu, S. Deng, et al., Mechanical stiffness of liver tissues in relation to integrin β1 expression may influence the development of hepatic cirrhosis and hepatocellular carcinoma, J. Surg. Oncol. 102 (2010) 482–489.
[167] C.-A. Lamontagne, M. Grandbois, PKC-induced stiffening of hyaluronan/CD44 linkage; local force measurements on glioma cells, Exp. Cell Res. 314 (2008) 227–236.
[168] W. Xu, R. Mezencev, B. Kim, L. Wang, J. McDonald, T. Sulchek, Cell stiffness is a biomarker of the metastatic potential of ovarian cancer cells, PLoS ONE 7 (2012) e46609.
[169] L. Bastatas, D. Martinez-Marin, J. Matthews, J. Hashem, Y.J. Lee, S. Sennoune, et al., AFM nano-mechanics and calcium dynamics of prostate cancer cells with distinct metastatic potential, Biochim. Biophys. Acta 1820 (2012) 1111–1120.
[170] A. Parekh, N.S. Ruppender, K.M. Branch, M.K. Sewell-Loftin, J. Lin, P.D. Boyer, et al., Sensing and modulation of invadopodia across a wide range of rigidities, Biophys. J. 100 (2011) 573–582.
[171] P.-H. Puech, K. Poole, D. Knebel, D.J. Muller, A new technical approach to quantify cell–cell adhesion forces by AFM, Ultramicroscopy 106 (2006) 637–644.
[172] K.J. Reeves, J. Hou, S.E. Higham, Z. Sun, J.P. Trzeciakowski, G.A. Meininger, et al., Selective measurement and manipulation of adhesion forces between cancer cells and bone marrow endothelial cells using atomic force microscopy, Nanomedicine (London) (2012) 921–934.
[173] M. Lekka, D. Gil, W. Dąbroś, J. Jaczewska, A.J. Kulik, J. Lekki, et al., Characterization of N-cadherin unbinding properties in non-malignant (HCV29) and malignant (T24) bladder cells, J. Mol. Recognit. 24 (2011) 833–842.
[174] H. Jin, J. Pi, X. Huang, F. Huang, W. Shao, S. Li, et al., BMP2 promotes migration and invasion of breast cancer cells via cytoskeletal reorganization and adhesion decrease: an AFM investigation, Appl. Microbiol. Biotechnol. 93 (2012) 1715–1723.
[175] C.E. McNamee, S. Armini, S. Yamamoto, K. Higashitani, Determination of the binding of non-cross-linked and cross-linked gels to living cells by atomic force microscopy, Langmuir 25 (2009) 6977–6984.
[176] S. Ramachandran, A.P. Quist, S. Kumar, R. Lal, Cisplatin nanoliposomes for cancer therapy: AFM and fluorescence imaging of cisplatin encapsulation, stability, cellular uptake, and toxicity, Langmuir 22 (2006) 8156–8162.
[177] C.E. McNamee, Y. Aso, S. Yamamoto, Y. Fukumori, H. Ichikawa, K. Higashitani, Chemical groups that adhere to the surfaces of living malignant cells, Pharm. Res. 24 (2007) 2370–2380.
[178] T. Sulchek, R. Friddle, T. Ratto, H. Albrecht, S. DeNardo, A. Noy, Single-molecule approach to understanding multivalent binding kinetics, Ann. N. Y. Acad. Sci. 1161 (2009) 74–82.
[179] X. Zhang, X. Shi, L. Xu, J. Yuan, X. Fang, Atomic force microscopy study of the effect of HER 2 antibody on EGF mediated ErbB ligand–receptor interaction, Nanomedicine (2012) 627–635.
[180] F. Braet, D. Vermijlen, V. Bossuyt, R. De Zanger, E. Wisse, Early detection of cytotoxic events between hepatic natural killer cells and colon carcinoma cells as probed with the atomic force microscope, Ultramicroscopy 89 (2001) 265–273.
[181] L. Chopinet, C. Roduit, M.-P. Rols, E. Dague, Destabilization induced by electropermeabilization analyzed by atomic force microscopy, Biochim. Biophys. Acta Biomembr. 1828 (2013) 2223–2229.
[182] P.A. Corsetto, A. Cremona, G. Montorfano, I.E. Jovenitti, F. Orsini, P. Arosio, et al., Chemical–physical changes in cell membrane microdomains of breast cancer cells after omega-3 PUFA incorporation, Cell Biochem. Biophys. 64 (2012) 45–59.
[183] A. Won, A. Ruscito, A. Ianoul, Imaging the membrane lytic activity of bioactive peptide latarcin 2a, Biochim. Biophys. Acta 1818 (2012) 3072–3080.
[184] J. Wang, Z. Wan, W. Liu, L. Li, L. Ren, X. Wang, et al., Atomic force microscope study of tumor cell membranes following treatment with anti-cancer drugs, Biosens. Bioelectron. 25 (2009) 721–727.
[185] P. Venkatesan, S. Das, M.M.R. Krishnan, C. Chakraborty, K. Chaudhury, M. Mandal, Effect of AEE788 and/or Celecoxib on colon cancer cell morphology using advanced microscopic techniques, Micron 41 (2010) 247–256.
[186] K.S. Kim, C.H. Cho, E.K. Park, M.-H. Jung, K.-S. Yoon, H.-K. Park, AFM-detected apoptotic changes in morphology and biophysical property caused by paclitaxel in Ishikawa and HeLa cells, PLoS ONE 7 (2012) e30066.
[187] X.-P. Wang, T.-S. Chen, L. Sun, J.-Y. Cai, M.-Q. Wu, M. Mok, Live morphological analysis of taxol-induced cytoplasmic vacuolization [corrected] in human lung adenocarcinoma cells, Micron 39 (2008) 1216–1221.
[188] C. Huang, H. Jin, B. Song, X. Zhu, H. Zhao, J. Cai, et al., The cytotoxicity and anticancer mechanisms of alterporriol L, a marine bianthraquinone, against MCF-7 human breast cancer cells, Appl. Microbiol. Biotechnol. 93 (2012) 777–785.
[189] Z. Bai, H. Zhang, L. Zhu, W. Zuo, J. Ye, J. Feng, et al., Lithium inhibits cell volume regulation by acting on chloride channels and modifies ultrastructures of the cell membrane in nasopharyngeal carcinoma cells, Eur. J. Pharmacol. 641 (2010) 88–95.
[190] J. Jiang, H. Jin, L. Liu, J. Pi, F. Yang, J. Cai, Curcumin disturbed cell-cycle distribution of HepG2 cells via cytoskeletal arrangement, Scanning (2012) 256–260.
[191] C. Ke, H. Jin, J. Cai, AFM studied the effect of celastrol on β1 integrin-mediated HUVEC adhesion and migration, Scanning (2012) 316–326.
[192] M. Taranta, A.R. Bizzarri, S. Cannistraro, Probing the interaction between p53 and the bacterial protein azurin by single molecule force spectroscopy, J. Mol. Recognit. 21 (2008) 63–70.
[193] S.E. Cross, Y.-S. Jin, Q.-Y. Lu, J. Rao, J.K. Gimzewski, Green tea extract selectively targets nanomechanics of live metastatic cancer cells, Nanotechnology 22 (2011) 215101.
[194] M. Targosz-Korecka, R. Biedron, A.M. Szczygiel, G. Brzezinka, J. Szczerbinski, A. Zuk, Stiffness changes of tumor HEp2 cells correlates with the inhibition and release of TRAIL-induced apoptosis pathways, J. Mol. Recognit. 25 (2012) 299–308.
[195] R. Kaul-Ghanekar, S. Singh, H. Mamgain, A. Jalota-Badhwar, K.M. Paknikar, S. Chattopadhyay, Tumor suppressor protein SMAR1 modulates the roughness of cell surface: combined AFM and SEM study, BMC Cancer 9 (2009) 350.
[196] M. Lekka, P. Laidler, M. Labedź, A.J. Kulik, J. Lekki, W. Zajac, et al., Specific detection of glycans on a plasma membrane of living cells with atomic force microscopy, Chem. Biol. 13 (2006) 505–512.
[197] S.E. Cross, Y.-S. Jin, J. Tondre, R. Wong, J. Rao, J.K. Gimzewski, AFM-based analysis of human metastatic cancer cells, Nanotechnology 19 (2008) 384003.
[198] M. Lekka, Atomic force microscopy: a tip for diagnosing cancer, Nat. Nanotechnol. 7 (2012) 691–692.
[199] M.F. Murphy, F. Lilley, M.J. Lalor, S.R. Crosby, G. Madden, G. Johnston, et al., Evaluation of a nonlinear Hertzian-based model reveals prostate cancer cells respond differently to force than normal prostate cells, Microsc. Res. Tech. 76 (2013) 36–41.
[200] I. Sokolov, V. Kalaparthi, M. Kreshchuk, M.E. Dokukin, On averaging force curves over heterogeneous surfaces in atomic force microscopy, Ultramicroscopy 121 (2012) 16–24.
[201] E. Dague, A. Delcorte, J.-P. Latge, Y.F. Dufrene, Combined use of atomic force microscopy, X-ray photoelectron spectroscopy, and secondary ion mass spectrometry for cell surface analysis, Langmuir 24 (2008) 2955–2959.
[202] N. Kodera, D. Yamamoto, R. Ishikawa, T. Ando, Video imaging of walking myosin V by high-speed atomic force microscopy, Nature 468 (2010) 72–76.

[203] G.E. Fantner, R.J. Barbero, D.S. Gray, A.M. Belcher, Kinetics of antimicrobial peptide activity measured on individual bacterial cells using high-speed atomic force microscopy, Nat. Nanotechnol. 5 (2010) 280–285.
[204] Y.F. Dufrêne, D. Martínez-Martín, I. Medalsy, D. Alsteens, D.J. Müller, Multiparametric imaging of biological systems by force–distance curve-based AFM, Nat. Methods 10 (2013) 847–854.
[205] A. Raman, S. Trigueros, A. Cartagena, A.P.Z. Stevenson, M. Susilo, E. Nauman, et al., Mapping nanomechanical properties of live cells using multi-harmonic atomic force microscopy, Nat. Nanotechnol. 6 (2011) 809–814.
[206] G. Longo, L. Alonso-Sarduy, L.M. Rio, A. Bizzini, A. Trampuz, J. Notz, et al., Rapid detection of bacterial resistance to antibiotics using AFM cantilevers as nanomechanical sensors, Nat. Nanotechnol. 8 (2013) 522–526.
[207] M. Stolz, R. Gottardi, R. Raiteri, S. Miot, I. Martin, R. Imer, et al., Early detection of aging cartilage and osteoarthritis in mice and patient samples using atomic force microscopy, Nat. Nanotechnol. 4 (2009) 186–192.
[208] N. Buzhynskyy, J.-F. Girmens, W. Faigle, S. Scheuring, Human cataract lens membrane at subnanometer resolution, J. Mol. Biol. 374 (2007) 162–169.
[209] N. Buzhynskyy, R.K. Hite, T. Walz, S. Scheuring, The supramolecular architecture of junctional microdomains in native lens membranes, EMBO Rep. 8 (2007) 51–55.
[210] M. Plodinec, M. Loparic, C.A. Monnier, E.C. Obermann, R. Zanetti-Dallenbach, P. Oertle, et al., The nanomechanical signature of breast cancer, Nat. Nanotechnol. 7 (2012) 757–765.
[211] E. Henderson, D.S. Sakaguchi, Imaging F-actin in fixed glial cells with a combined optical fluorescence/atomic force microscope, NeuroImage 1 (1993) 145–150.
[212] J. Vesenka, C. Mosher, S. Schaus, L. Ambrosio, E. Henderson, Combining optical and atomic force microscopy for life sciences research, BioTechniques 19 (1995) 240–248(849, 852–853).
[213] S. Fukuda, T. Uchihashi, R. Iino, Y. Okazaki, M. Yoshida, K. Igarashi, et al., High-speed atomic force microscope combined with single-molecule fluorescence microscope, Rev. Sci. Instrum. 84 (2013) 073706.
[214] A. Colom, I. Casuso, F. Rico, S. Scheuring, A hybrid high-speed atomic force–optical microscope for visualizing single membrane proteins on eukaryotic cells, Nat. Commun. 4 (2013).
[215] Y. He, M. Lu, J. Cao, H.P. Lu, Manipulating protein conformations by single-molecule AFM-FRET nanoscopy, ACS Nano 6 (2012) 1221–1229.
[216] Q. Wang, M. Wang, S. Li, X. Xing, X. Liu, S. Dong, et al., AFM detection of mitogen-induced morphological changes in human B lymphocyte, Scanning 34 (2012) 60–67.