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Review

Nanomechanics of lipid bilayers by force spectroscopy with AFM: A perspective

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ABSTRACT

Lipid bilayers determine the architecture of cell membranes and regulate a myriad of distinct processes that are highly dependent on the lateral organization of the phospholipid molecules that compose the membrane. Indeed, the mechanochemical properties of the membrane are strongly correlated with the function of several membrane proteins, which demand a very specific, highly localized physicochemical environment to perform their function. Several mesoscopic techniques have been used in the past to investigate the mechanical properties of lipid membranes. However, they were restricted to the study of the ensemble properties of giant bilayers. Force spectroscopy with AFM has emerged as a powerful technique able to provide valuable insights into the nanomechanical properties of supported lipid membranes at the nanometer/nanonewton scale in a wide variety of systems. In particular, these measurements have allowed direct measurement of the molecular interactions arising between neighboring phospholipid molecules and between the lipid molecules and the surrounding solvent environment. The goal of this review is to illustrate how these novel experiments have provided a new vista on membrane mechanics in a confined area within the nanometer realm, where most of the specific molecular interactions take place. Here we report in detail the main discoveries achieved by force spectroscopy with AFM on supported lipid bilayers, and we also discuss on the exciting future perspectives offered by this growing research field.

Contents

1. Introduction

The diversity in the chemical composition of the ample phospholipid repertoire is intricately related to biomembrane function [1,2]. In addition to creating a molecular scaffold that acts as a semi-permeable barrier that separates the extra- from the intracellular environment, lipids play multiple roles in cell processes; for example, lipids provide membranes with the potential for budding, tubulation, fission and fusion [2]. From the mechanical perspective, lipid bilayers not only determine the molecular architecture of cells, which are known to naturally perform their function under the effect of a complex combination of forces [3], but also play a pivotal role in tuning the function of several membrane proteins [4]. In particular, the chemical composition of lipid bilayers has shown to decisively activate the gating properties of several ion and mechanosensitive channels by creating the 'correct' chemical environment [5–8] or by adjusting the membrane intrinsic curvature under mechanical stress [9,10], respectively. Such local mechanochemical adjustment of the membrane is also

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crucially related to the correct folding [11] and aggregation processes [12] of membrane proteins [13]. Therefore, there are tantalizing evidences about the subtle interplay between the local lipid chemical composition, the membrane lateral organization, which defines its 'mechanical state' and compliance, and the overall membrane function.

Several techniques have been employed to quantitatively investigate the mechanical properties of lipid bilayers. For example, photoelectron correlation spectroscopy [14], dynamic light scattering [15] and also cryoelectron microscopy [16] have been used to study the behaviour of vesicles under osmotic stress. The most outstanding approach, the micropipette aspiration technique [17,18], has provided a wealth of information regarding quantitative values for membrane elastic moduli for area dilation, shear and bending, and also for interbilayer friction [19,20]. While crucial, these experiments are mostly restricted to the use of giant vesicles, thus providing a 'mesoscopic' outlook on bilayer mechanical stability. At the nanometer scale, the surface force apparatus (SFA) has provided valuable insight into the study of lipid bilayer adhesion, fusion and healing, and allows direct measurement of the interaction forces arising between symmetrical and asymmetrical lipid bilayers [21–24]. However, this technique is mostly limited to the study of the interaction between two facing bilayers, which are deposited onto the two opposing flat mica surfaces. Therefore, the independent measurement of the mechanical properties of a single bilayer requires other experimental tools, with enhanced spatial resolution. Atomic force microscopy (AFM) has emerged as an essential tool to investigate the topology of lipid bilayers, allowing for the study of both, static and dynamic processes [25–27]. These studies, mainly conducted on lipid monolayers and supported lipid bilayers (SPBs), have provided a wealth of new information regarding surface topology in liquid environment with nanometer resolution [28–30]. Besides the impressive progress on the study of the topographic properties of supported lipid bilayers, the AFM has also been used in its force spectroscopy mode to gain insights into their mechanical properties. From the pioneering measurements by Dufrene and colleagues [31,32], the AFM experiments in force spectroscopy mode have allowed to gain insights into the nanomechanical properties of lipid membranes at the nanometer/nanonewton scale in a wide variety of systems. In particular, these measurements have provided direct measurement of the molecular interactions arising between neighboring phospholipid molecules and between the lipid molecules and the surrounding solvent environment.

In this review, we aim at reporting the main discoveries that these measurements have elicited and the future perspectives that this emergent research field offers in the near future. All in all, the goal is to illustrate how these novel experiments have provided a new perspective on membrane mechanics in a confined area within the nanometer realm, where most of the specific molecular interactions take place [33–36].

2. The need of a well-defined molecular fingerprint

Force spectroscopy with AFM has revealed an unprecedented amount of information regarding the mechanical properties of a great variety of systems, ranging from indenting solid hard materials [37] to pulling soft individual molecules such as proteins [38], sugars [39] or DNA [40]. Taken together, the strength of these experiments is that they allow direct measurement of the interaction forces when a finite and small number of molecules are involved. In all these measurements, most of them conducted under typical constant velocity conditions, the deflection of the cantilever tip is recorded as a function of the distance between the AFM probe and the sample. Due to the different stiffness of the tested systems, the spring constant of the cantilever has to be adequately chosen in order to match the stiffness of the probed sample. This is a crucial step to achieve the correct sensitivity in the force measurement. For example, in the case of probing the mechanical properties of hard substrates such as silicon or graphite, cantilevers with high spring constants ($k_c \sim 300$ N/m) are required, since the applied forces required to test the onset of plastic deformation of such materials is in the order of the micronewton (μN) [41]. By contrast, unfolding a single protein molecule with force requires only the application of a pulling force of a few tens of piconewtons (pN) [42]. In that case, soft cantilevers of $k_c \sim 10$ pN/nm are required. The individual spring constants of the used cantilever are individually calibrated, typically using the equipartition theorem [43]. Within this large spectrum of forces that can be accurately measured with the AFM, ranging from the micronewton to the piconewton, the stiffness of lipid bilayers occupies an intermediate position; the force required to trigger an elastic–plastic transition on a lipid bilayer occurs in the nanonewton (nN) regime [31].

A schematic picture of the typical AFM set-up used in the indentation experiments on supported lipid bilayers is shown in Fig. 1. Once a bilayer has been identified by means of AFM imaging, a set of different force curves are conducted in the center of the islands. Applying a constantly increasing force with an AFM cantilever tip on a lipid bilayer results in a typical force curve such as the one shown in Fig. 1f. When the cantilever tip is away from the bilayer sample, no interaction between the tip and the bilayer is observed (Fig. 1c). As the tip approaches the sample, the short-range interactions between tip and sample start being measurable. These interaction forces have a varying origin, ranging from Derjaguin–Landau–Verwey–Overbeek (DLVO) forces, hydration forces or steric forces. The magnitude and nature of these forces is highly dependent on the bilayer chemistry, the physicochemical properties of the measuring buffer solution and the chemistry of the AFM probe. In general, the combination of these forces extends up to a few hundred of piconewtons. On further approaching the cantilever tip to the bilayer sample, they become in mechanical contact. Upon further increasing the pushing force, the membrane elastically deforms (Fig. 1d). This is confirmed by the observation that, upon retracting the cantilever from this force point, no plastic event is observed. When the applied force reaches a threshold value, a well-defined jump in the force-distance curve is observed (Fig. 1e). This jump measures ~4 nm, which correlates well with the height of the bilayer as revealed by AFM images. Such breakthrough event, which typically occurs at several nanonewtons of force, marks the penetration of the cantilever through the lipid bilayer, identifying the onset of the plastic deformation regime. Therefore, the breakthrough force unequivocally identifies the maximum force that the membrane is able to withstand before being indented by the cantilever tip. Crucially, the determination of the breakthrough force within sequential force measurements is highly reproducible. Therefore, even for different days of experiments, the distribution of breakthrough forces is mainly dependent on the error in the calibration of the spring constant of the cantilever, which is around 15–20% when using the equipartition theorem. Therefore, akin to the unfolding force in single protein mechanical experiments [38], the transition force between double stranded DNA to single stranded DNA [40], the chair-boat transition force observed when stretching sugar chains [39] or the force required to indent hard materials such as alkali halide single crystals [41], the breakthrough force value unambiguously fingerprints the (nano)mechanical resilience of the probed membrane.

Such a breakthrough in the force-distance curves has been identified for a variety of thin films when placed under an applied force. Since it was first reported by Ducker and Clarke upon penetration of a zwitterionic surfactant bilayer [44], the observation of such discontinuity in the force curves has been crucial to understand the ordering mechanisms of surface-supported monolayers [45–47]. Furthermore, film rupture has been also observed in confined liquids, corresponding to the layer-by-layer tip penetration through the well-ordered squeezed liquid film [48–53]. Regarding lipid bilayers, the first reported breakthrough event, correlated with the tip penetration through the bilayer, was observed on DSPE, DGDG and DOPE bilayers by

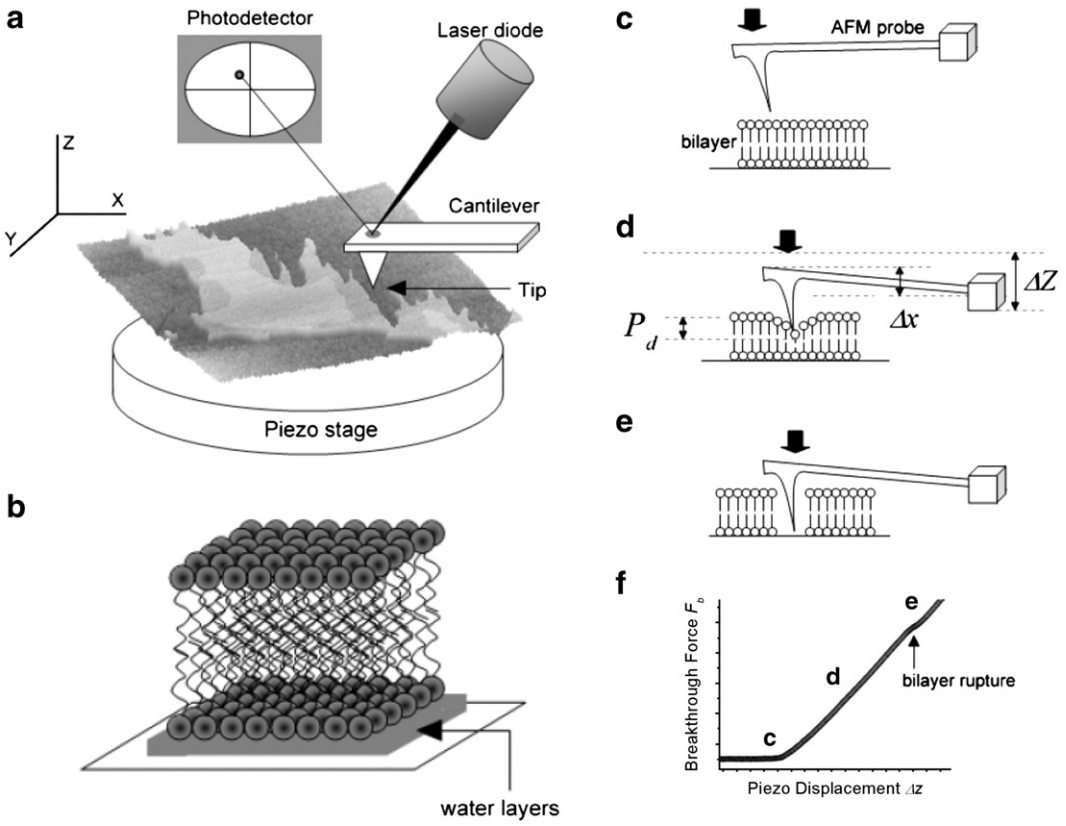

Fig. 1. Probing the mechanical stability of a lipid bilayer with an AFM tip. (a) Schematics of the experimental AFM set-up. In our experiments, micrometer-size islands of supported lipid bilayer are first identified using conventional imaging of the surface. Force curves are then conducted in the center of such bilayers. (b) Schematics of the lipid bilayer. (c–e) Schematics of the indentation process on a lipid bilayer using an AFM cantilever tip. Snapshots corresponding to the moment where the cantilever tip and the bilayer are not interacting (a), the bilayer is elastically deformed by the cantilever tip (b), and the tip ruptures the lipid bilayer, thus becoming in contact with the mica substrate. (f) Experimental indentation trace on a DMPC bilayer, showing the different scenarios exemplified by the cartoons (c–e).

Dufrene et al. [31]. Since such pioneering work, many studies have reported the presence of a breakthrough in the indentation curves of lipid bilayers composed of different phospholipid molecules. Despite these meritorious earlier proof-of-principle observations, a quantitative measurement of the force at which the jump occurs has not been conducted until recently. Indeed, the quantification of the force value at which the jump takes place represents a direct measurement of the membrane mechanical stability at the nanometer scale. The clean, unambiguous determination of the breakthrough event allows clear separation of the elastic from the plastic regime in the bilayer deformation process [54]. The earlier reports on the breakthrough events in lipid bilayers were mostly directed at complementing AFM topography studies; identifying the yield threshold force allowed to place an upper limit to the maximum set-point force applied to the bilayer in order to obtain enhanced topographic resolution without damaging the substrate [32]. By contrast, recent works have been exclusively focused on the precise, systematic measurement of the threshold force on a variety of chemically distinct lipid bilayers as a straightforward experimental way to gain direct insight into the relationship between membrane conformation and membrane nanomechanical properties. In particular, the latter studies have succeeded in directly relating the effect of the ionic strength of the measuring solution [55], the effect of temperature [56,57], the effect of an electric field [58,59] and of phase separation [60] on the nanomechanics of a variety of chemically distinct lipid bilayers.

3. The local interaction forces between the AFM probe and the lipid bilayer

A typical force spectroscopy experiment relies on the presence of a lipid bilayer that extends some hundreds of nanometers or even micrometers to ensure that the surface is homogenous and defect-free. Typically, supported lipid bilayers are prepared from liposome deposition [26] or from Langmuir–Blodgett two-step deposition transfer [61]. In general, high ionic strength conditions enhance the deposition of liposomes onto hydrophilic surfaces such as mica, allowing for larger and more homogenous islands [55]. The presence of extended bilayers is typically certified by imaging the surface before the force experiments are conducted. In principle, the central areas of the bilayer islands give rise to more reproducible results and higher values of breakthrough forces than the measurements performed in the perimeter or rims of the bilayer [62]. Interestingly, the chemistry of the hydrophilic supporting substrate where the bilayers are adsorbed does not significantly affect the yield threshold determination [63]. By contrast, the chemistry of the AFM tip has shown to have a significant effect on the measured nanomechanics of the measured supported bilayers [30]. In general, typical experiments are performed with hydrophilic tips, either using bare silicon nitride tips or chemically modified tips with an alcohol termination, –OH. However, when using hydrophobic terminations, e.g., $-CH_3$, Schneider et al. showed that the breakthrough force vanishes for a variety of phospholipids, thereby occurring essentially on contact [30]. Furthermore, when bare silicon nitride tips are used, especially under high ionic strength conditions, a second bilayer can form on the cantilever tip apex. Upon approaching the 'sample' bilayer, the force plots exhibit two consecutive breakthrough events [55,64,65]; in these cases, the resulting force plot recordings are not taken into account for data statistics. However, such approach proves as a good experimental strategy to locally probe bilayer–bilayer interactions. For example, the mechanical stability of two interacting DOPS bilayers showed to significantly increase upon introducing synapsin I in the measuring

solution. In this case, the two facing bilayers were so stiff that they could not be penetrated by the AFM tip [65].

As stated above, most of the studies regarding the nanomechanics of lipid bilayers, especially the most recent ones, concentrate on the quantitative determination of the yield threshold force. However, prior to the plastic failure, a variety of interaction forces arising between the cantilever tip and the phospholipid surface can be detected. This is schematically exemplified in Fig. 2a and b. The first important contribution of the interaction between the phospholipids

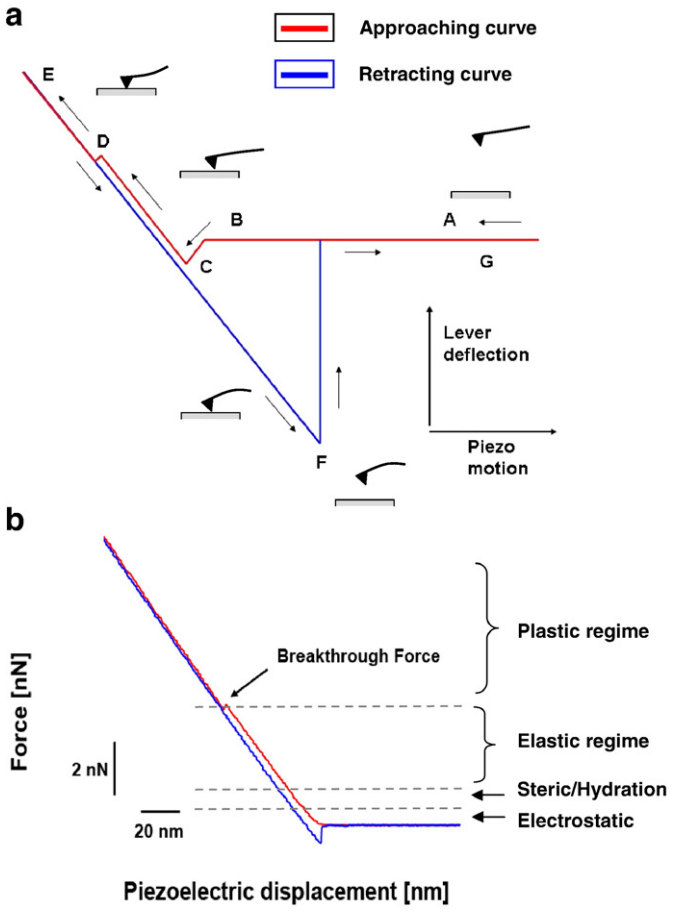

Fig. 2. The force curves on a supported lipid bilayer exhibit a well-defined jump of ~4 nm, a true molecular fingerprint that marks the penetration of the AFM tip through the lipid bilayer. (a) Schematics of a force plot on a supported lipid bilayer; at long distances (A) the cantilever and the sample are far apart, and therefore no cantilever deflection is measured. Upon approaching, the cantilever starts feeling the long-range interactions, mainly of electrostatic and van der Waals origin (B). If the bilayer and the cantilever tip are equally charged, a jump to contact occurs (C). Otherwise, if they have the same electric charge, repulsion is observed instead. Further approaching the cantilever tip onto the bilayer results in the deflection of the cantilever as it is physically in contact with the membrane. At moderate pushing forces, the cantilever experiences a combination of hydration and steric forces. After this point, the membrane deforms elastically, until a critical yield force is reached (D); at this force value, the jump of the cantilever through the membrane occurs, which is a signature of the maximum force that the membrane is able to withstand before breaking. Further compression of the cantilever onto the substrate (typically mica) results in further cantilever deflection. The retracting part of the cycle may show hysteresis, since the cantilever tip gets in some cases adhered to the surface, depending on the bilayer and tip chemistries, until a critical adhesion force is achieved (F). Once the cantilever overcomes the adhesion force, the cantilever detaches from the surface (G). (b) Typical force curve on a DOPC lipid bilayer. In a typical cycle, the cantilever tip approaches the bilayer (red trace) up to a set maximum force. The piezoelectric is subsequently retracted (blue trace) at the same velocity. During the approaching part of the cycle, the cantilever tip applies an increasing *pushing* force on the bilayer. The cantilever first probes the electrostatic and steric/hydration forces that act on the bilayer. The bilayer is then elastically deformed, until a sudden jump appears, which marks the plastic penetration of the cantilever tip through the lipid bilayer. The force value at which such a breakthrough event occurs (arrow) is strongly dependent on the chemical composition of the lipid bilayer.

and the cantilever tip is electrostatic in origin [66]. In general, the majority of phospholipid molecules are negatively charged, even those that are zwitterionic, as revealed by zeta potential measurements [55]. On the other hand, the silicon nitride tips used to perform force spectroscopy experiments are known to be slightly negatively charged at neutral pH [63]. Using the DLVO theory, which combines electrostatic with Van der Waals interactions, the maximum interaction repulsive force between the negatively charged surface and the negatively charged tip contributes to up to a few pN, due to the small charge density of the silicon nitride tip (Fig. 2b). Such electrostatic contributions can be experimentally tested by functionalizing the AFM tip with carboxyl termination. In this case, when the pH is higher than the cantilever pK_a, both the bilayer surface and the tip surface are negatively charged, and hence a long-range electrostatic repulsion can be observed, expanding up to ~300 pN [67]. Indeed, such tip functionalization strategy has successfully been used to conduct a chemical titration on supported lipid bilayers using force spectroscopy [67]. Furthermore, hydration and steric forces have been described as responsible for short-distance repulsion between two opposing lipid bilayers. Indeed, it is the force required to remove the water molecules adsorbed on amphiphilic groups as both surfaces come into contact [68]. In this vein, it has been shown that the role of water is crucial for membrane stability. The short-range hydration force, that decays exponentially with the distance, has been reported to extend up to 5 nN [31] (Fig. 2b). Therefore, neither the electrostatic nor the steric–hydration forces can account for the force-distance curves acquired with the AFM until the breakthrough point, which typically occurs at several nanonewtons. Instead, a mechanical contribution concerning the elastic deformation of the membrane is required in order to fully account for the force-distance shape of the curve until the plastic regime was achieved (Figs. 1d and 2b). While Dufrene et al. [31] proposed the Hertz relationship as a suitable model to reproduce the experimental data, Garcia-Manyes et al. showed that in their experiments, the Hertz model failed to reproduced the experimental data: instead, a simple elastic model built up by parallel springs reproduced quite well the experimental data [55]. The failure of the Hertz model to reproduce the experimental data has been also reported by Leonenko et al. [57]. Once the energy applied by the cantilever tip overcomes the lateral elastic interactions defining the supported bilayer, the instability in the force plot is observed. Such breakthrough event separates the elastic from the plastic regime (Figs. 1e and 2a(D) and arrow in Fig. 2b). Since the work exerted by the cantilever is done over very short distances, the rupture of a lipid bilayer can be regarded as a brittle process.

In a set of three interconnected papers [33–35], Butt and coworkers presented a seminal theory that described the film rupture observed in lipid bilayers that relate microscopic parameters with measurable quantities. The model is based on the assumption that the tip breaks through the lipid bilayer after the formation of a sufficiently big hole under the tip. It predicts that the breakthrough force increases with increasing approach speed of the tip. The average activation volume is about the volume of a single lipid molecule. Furthermore, the authors described two complementary models to explain the two-state film rupture reaction. In the first one, the continuum nucleation theory, the distribution of forces required to create a hole in the membrane is connected with the line tension, Γ, which represents the free energy associated with the unsaturated bonds of the molecules at the periphery of the hole, and with the effective spreading pressure, S, which is a parameter relevant for membrane adsorption to a solid surface. In the second model, the molecular model, each molecule in the film has certain binding sites, which are energetically favorable positions. To jump from a binding site to an adjacent free binding site, a potential energy barrier has to be overcome. In the presence of an AFM tip that presses the film, it becomes energetically favorable for the molecules to jump to the side and form a hole in the center of the tip. The film then ruptures and the tip penetrates. Both theories, namely

the nucleation and the molecular, reproduced well the experimental data. The theories presented in these pioneering studies have already helped interpreting a variety of recent works [60,69].

In earlier experiments on indentation of lipid membranes with the AFM tip, the identification of the rupture force was essentially useful to define the tip-sample separation distance while imaging the supported lipid bilayers [32]; working at set-point forces lower than the yield threshold ensured images with high resolution. Conversely, working at higher forces resulted in the damage of the membrane, and therefore, in a lack of imaging resolution [61]. In a series of follow-up experiments, the presence of the breakthrough force identified by the normal force measurements has been related to friction (or lateral force) experiments. As a whole, these experiments concluded that the friction and normal force curves are interrelated. In particular, Grant and Tiberg studied the friction properties of DOPC [70], highlighting its capability to resist substantial normal loads and its success in providing an efficient role as a lubricant. An interesting work by Benz and coworkers investigated the friction properties of a DPPE/DLPE lipid bilayer with AFM and with surface force apparatus [21], highlighting the correlation of the results obtained by both complementary techniques. In such a work, the authors concluded that the appearance of single defects in lipid bilayers, which can be detected with the AFM thanks to its high spatial resolution, could be related to the averaged stability of the bilayer as measured by SFA [24]. A recent report by Trunfio-Sfarghiu et al. has investigated the role of phospholipid bilayers in controlling and reducing frictional forces between biological surfaces [71]; in their study, the authors combined the AFM force spectroscopy mode with a tribometer to study the relationship between the mechanical resistance to indentation and the frictional forces exhibited by a DOPC bilayer. According to their results, the friction coefficient is correlated with the force needed to penetrate the bilayer with the AFM tip, in the sense that surfaces covered with bilayers exhibiting a stronger mechanical resistance to AFM tip indentation showed lower and more stable friction coefficients. Finally, Oncins et al. conducted lateral force microscopy studies with the AFM on a DMPC bilayer with varying amounts of NaCl in the measuring buffer solution [72]. The authors discovered that the presence of Na^+ cations in the solution induced significant structural changes in the DMPC bilayer. This observation translated into three different friction regimes observed as the vertical force exerted by the tip on the bilayer increased. All in all, the already extensive literature in the field highlights the success of force spectroscopy AFM on lipid bilayers in providing insight into the local lateral forces that act between neighboring phospholipid molecules at the nanometer scale. These studies also highlight the power of force spectroscopy at investigating the mechanical properties of single lipid membranes in a confined area.

4. Effect of ionic strength on the mechanical stability of lipid bilayers

The role of divalent cations was long known to have an effect on the packing properties of lipid bilayers. By contrast, the role of monovalent ions on the bilayer structure has been for a long time underestimated. Despite this prevailing view, recent molecular dynamics simulations showed that not only divalent cations such as Mg^{2+} and Ca^{2+} [73], but also monovalent cations such as Na^+[74], would have an important effect on bilayer packing. These revealing simulations triggered the experimental study reported by Garcia-Manyes et al., where they conducted a quantitative force spectroscopy study on model DLPC, DMPC and DPPC membranes and also on natural *E. coli* membranes to probe the effect of ionic strength on the (nano)mechanical properties of the bilayers [55]. Ionic strength enhances the deposition of lipid bilayers on hydrophilic substrates [63]. The trend is observed in Fig. 3a and b, where two contact mode 5×5 μm^2 images of a DMPC bilayer in the absence (Fig. 3a) and presence (Fig. 3b) of ionic strength are

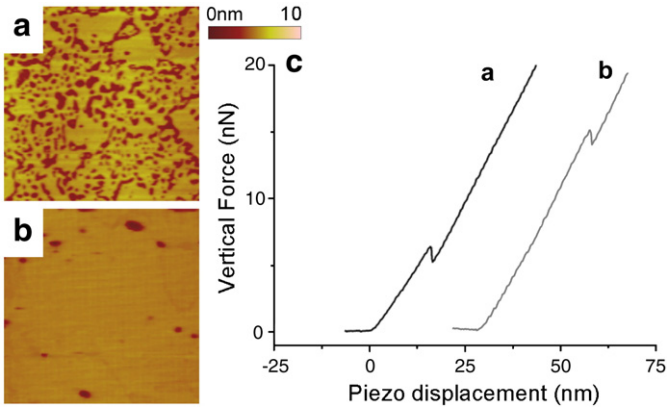

Fig. 3. The deposition process and the mechanical stability of a DMPC bilayer are highly dependent on the ionic strength of the measuring solution. (a) AFM 5×5 μm^2 contact mode image of a DMPC bilayer on mica in 10 mM HEPES, pH 7.4. (b) AFM 5×5 μm^2 contact mode images of a DMPC bilayer on mica in a 10 mM HEPES, 150 mM NaCl + 20 mM $MgCl_2$, pH 7.4 buffer. (c) Force curves performed on sample (a) (black line) and on sample (b) (grey line). In the absence of ionic strength in the solution, the DMPC bilayer breaks on average at forces ~4 nN, whereas under high ionic strength conditions the bilayer breaks at forces as high as ~15 nN.
Adapted from [55].

shown. As it can be observed, higher ionic strength induces a better and faster deposition of the bilayer onto the negatively charged mica surface. From the mechanical viewpoint, the force required to puncture the bilayer in the absence of ionic strength (~4 nN) is significantly lower than that required to pierce the DMPC bilayer with an increasing concentration of Na^+ in the measuring solution [55]. Upon including divalent ions such as Mg^{2+}, the mechanical stability of the membranes is further enhanced, as demonstrated by the much higher breakthrough force (~15 nN) required to indent a DMPC bilayer in the presence of 150 mM NaCl + 20 mM $MgCl_2$, as it is shown in Fig. 3c. This experimental study was based on the detection of the yield threshold force from a quantitative and statistically relevant perspective, highlighting the role of the breakthrough force, not only as a fingerprint for chemically distinct phospholipids, but also for the same phospholipid bilayer in different experimental environments.

5. Effect of temperature on the nanomechanics of lipid bilayers

Natural membranes are mainly composed of phospholipids with a high content of chain unsaturations. The phase transition of these membranes occurs at low temperatures, which induces fluidity under physiological conditions. A phase transition process involves a cooperative rearrangement of the individual phospholipid molecules that compose the bilayer. Such phase transition involves a change in the packing properties of the overall bilayer, thereby greatly affecting the lateral interactions between neighboring molecules. Such a variation in the lateral interactions at the molecular level is likely to have a direct reflection into the mechanical properties of the bilayer. Indeed, based on earlier experiments, it seemed to be controversial whether the breakthrough event occurs only in the solid-like gel phase [61] or also in the liquid-like state [75]. In this framework, Leonenko et al. first demonstrated that, for a DPPC membrane, the breakthrough force decreases as the temperature increases and that at elevated temperatures DPPC shows similar instability in the force plot as fluid-phase DOPC and DOTAP bilayers [57]. However, a quantitative measurement of such phase-induced change in the mechanical stability of the bilayer remained elusive. Using a combination of temperature-controlled AFM imaging mode with force spectroscopy, Garcia-Manyes et al. showed that the force needed to pierce the lipid bilayer is temperature-dependent, and that the solid-like phase exhibits a much higher yield threshold force than its liquid-like counterpart [56]. These experiments, conducted on DPPC, DMPC and DLPC bilayers, showed that, upon

increasing the temperature of the system, two distinct phase transitions could be indentified. This is observed in the temperature-controlled AFM contact mode images on a DMPC bilayer shown in Fig. 4a–e. In these images, the temperature is increased from 19 °C (Fig. 4a) up to 37.5 °C (Fig. 4e). At low temperatures (Fig. 4a) the bilayer is in the gel phase. Upon increasing the temperature, the bilayer undergoes a first phase transition (Fig. 4b) that is completed at 30.3 °C (Fig. 4c). Upon further increasing the temperature, the bilayer undergoes a second phase transition (Fig. 4d) that is completed at 37.5 °C (Fig. 4e). The whole temperature range encompassing both transitions extended ~15 °C, therefore presenting a much broader transition than that measured by e.g. DSC techniques. Typical force curves obtained at three different temperatures (19 °C, 30.3 °C and 37.5 °C, respectively) are shown in Fig. 4f. The global trend, relating the breakthrough force at each particular temperature is shown in Fig. 4g. Within the solid-like phase, the threshold force decreases as the temperature increases, and suddenly drops as it approaches the melting temperature, T_M. Interestingly, a well in the yield force vs. temperature plot occurs around T_M, thus providing a signature of an "anomalous mechanical softening" of the bilayer around temperatures close to the phase transition. Indeed, around the chain melting temperature, the phospholipids exhibit pronounced changes in elastic constants, e.g., compressibilities, bending elasticity and relaxation times, due to fast fluctuations in volume and area [76]. In Fig. 4g, the area marking the temperature range encompassing the two phase transitions observed with the AFM images is shaded in grey. Similar results were obtained in the case of DPPC supported membranes, although the phase transition occurred at higher temperatures [56].

Two different interpretations could eventually explain the presence of the two distinct transitions observed in the AFM experiments; a first interpretation could involve a first phase transition from the gel phase to the liquid phase with highly ordered structure, and a subsequent transition from such ordered liquid phase to a liquid disordered phase. The second interpretation, also suggested by Keller et al., would involve the individual melting of the DPPC leaflets that compose the bilayer [77]. The low temperature transition would be related to the melting of the leaflet that is far from the surface (distal leaflet), whereas the second transition would involve the phase transition of the leaflet in contact with the mica surface (proximal leaflet).

In order to resolve such a dichotomy, Oncins et al. followed the topographic and mechanical evolution of a single DPPC monolayer with temperature, revealing the presence of a single phase transition event [78]. This finding was a direct experimental proof that the two phase transitions observed in the counterpart DPPC bilayer correspond to the individual phase transition of the two leaflets composing the bilayer. Akin to the measurements for lipid bilayers, the force value required to penetrate the lipid monolayer was measured to be highly dependent on the temperature and on the phospholipid phase, ranging from 120 pN at room temperature (gel phase) to 49 pN at 65 °C (liquid phase). Surprisingly, the force value required to penetrate a monolayer represents a two orders-of-magnitude decrease with respect to the forces required to indent a DPPC bilayer, which is in the order of several nanonewtons, spanning from ~23 nN in the gel phase at 25 °C to ~6 nN in the liquid phase at 63 °C [56]. This is observed in Fig. 5, where two typical traces corresponding to a DPPC bilayer in the gel phase and to its monolayer counterpart are shown. The strikingly different mechanical stability measured for both the DPPC bilayer and monolayer highlights the increased complexity in the interaction forces that keep neighboring molecules together in the case of lipid bilayers as compared to the related monolayers. These surprising results certainly invite further investigation. A possible partial source of interpretation is that, in the case of lipid monolayers, the phospholipid headgroups are facing the hydrophilic mica substrate, while the apolar chains are facing the solution. Similar experiments conducted on monolayers deposited on a hydrophobic substrate (e.g. highly ordered pyrolytic graphite, HOPG), where the hydrophilic headgroups will be facing the AFM tip, will complement the results reported in [78] and allow direct comparison between the mechanical stability of lipid bilayers with their monolayer counterparts.

A recent, similar experiment dealing with the temperature-dependence of the mechanical stability of POPE bilayers further

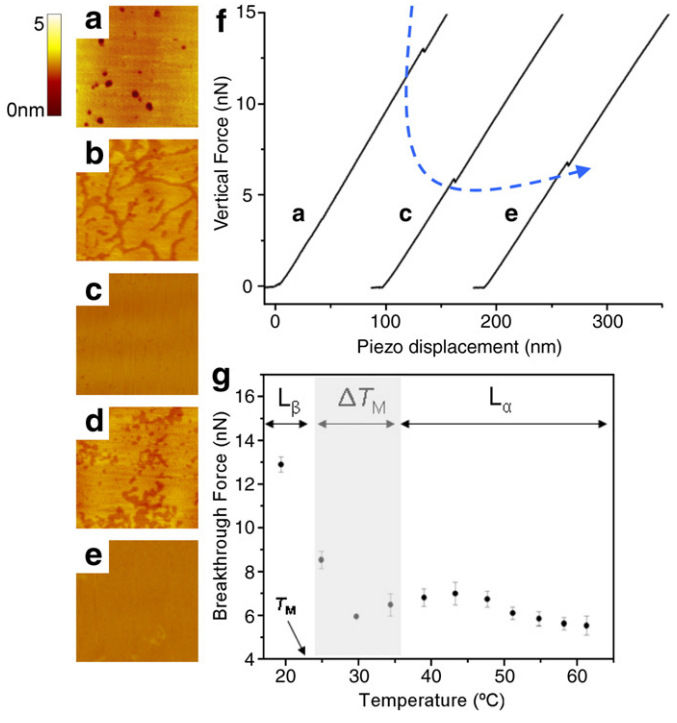

Fig. 4. The mechanical stability of a DMPC bilayer captures the gel-liquid phase transition. (a–e) AFM 5×5 μm² contact mode images showing the phase transition for a DMPC-supported bilayer upon heating the sample (a) 19 °C, (b) 27.2 °C, (c) 30.3 °C, (d) 31.3 °C, and (e) 37.5 °C. Reproduced from [56]. (f) Evolution of the breakthrough force with temperature, exhibiting a minimum at temperatures around 30 °C. (g) Dependence of the breakthrough force with temperature, resulting in a 'well' in the plot at temperatures close to the phase transition (dark area) as a result of a 'mechanical softening of the bilayer'. Reproduced from [56].

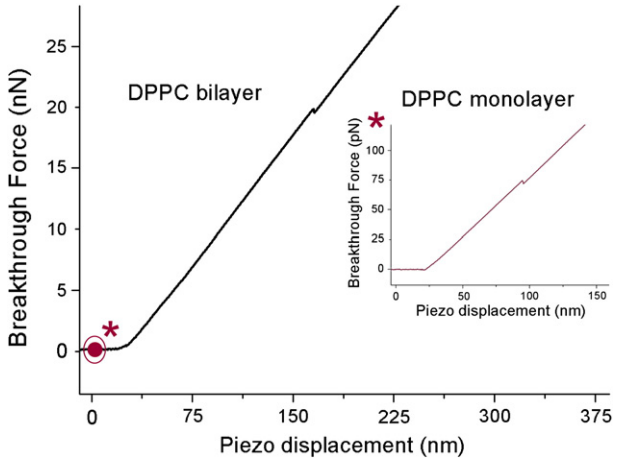

Fig. 5. The mechanical stability of a DPPC bilayer is dramatically higher than that of a DPPC monolayer. Force vs. piezo displacement curve of a DPPC bilayer at room temperature (gel phase) under high ionic strength conditions, exhibiting a breakthrough force of ~20 nN. Inset: Force vs. piezo displacement of the analogous DPPC monolayer in the gel phase, exhibiting the breakthrough event at ~75 pN. The asterisk symbol in the force plot corresponding to the bilayer marks the force range where the indentation process occurs for the DPPC monolayer, thus highlighting the huge disparity in the mechanical stability between a DPPC bilayer [56] and a DPPC monolayer [78] at the same temperature.

highlights the success of yield threshold force measurement to fingerprint phase separation in lipid bilayers. In particular, POPE undergoes two distinct phase transitions, the first one involving the transition from gel to liquid crystalline phase (L_α), and the second one involving the formation of intermediate structures or stalks in the transition from L_α to inverted hexagonal phase, H_{II}, exhibiting higher mechanical stability [79]. Overall, the combination of force spectroscopy experiments with temperature control demonstrates the ability of force spectroscopy with AFM to individually identify and characterize the different molecular conformations determining each distinct phase of a supported phospholipid bilayer from a nanomechanical viewpoint.

6. Quantitative determination of the yield threshold force value permits identification of phase separation in lipid bilayers composed of mixtures of phospholipids

In light of the results of the works reported through the years and reviewed here, it seems now clear that the force at which a phospholipid bilayer breaks is a direct signature of the chemical composition and molecular organization of a particular bilayer in a precise buffered environment at a well-defined temperature. Therefore, these studies can be now expanded to more complex systems composed of a mixture of different phospholipids with varying compositions. Such complex systems are likely to separate into domains of nanometric dimensions that are difficult to indentify and distinguish by common ensemble techniques. In this vein, Chiantia et al., in an effort to study phase separation in a raft-exhibiting DOPC/SM/Chol mixture, nicely showed how the force required to indent the bilayer present in the liquid ordered phase (L_o) is significantly greater (10.2 nN) than the force required to puncture the same bilayer in the liquid disordered phase, (L_d), 6.5 nN [60]. In a similar approach, Sullan et al. observed that, upon addition of ceramide, the mixture composed by DOPC/SM/Chol increases its mechanical stability, both in the liquid ordered and liquid disordered phases [80]. A third recent report on POPE/POPG (3:1, mol/mol) mixtures made use of force spectroscopy to identify the nature of two different calcium-induced existing domains observed by AFM images [81]. The distinct distribution of breakthrough forces revealed that the higher domains showed a higher mechanical stability (0.92 nN), while the lower domains exhibited a lower mechanical stability of 0.24 nN. These measurements allowed correlating the mechanically stable domains with the gel phase (L_{β}), and the mechanically labile domains with the fluid (L_α) phase. Altogether, these recent experiments illustrate the power of performing force spectroscopy on lipid bilayers composed of a mixture of phospholipids, thus providing a first step towards reconstruction and understanding of natural membranes, which are composed of a great variety of different chemically distinct lipid molecules.

7. Perspective and future

AFM force spectroscopy on lipid bilayers has already established itself as an emergent new tool to explore the mechanical properties of supported lipid bilayers with nanometer and piconewton resolution. The interaction of the sharp apex of an AFM tip with the surface of a well-deposited lipid bilayer has allowed to probe the nature of the different forces involved in the tip-sample interface. Moreover, it has permitted identification of the intermolecular forces that keep individual phospholipid molecules together. The first reported works in the field used the breakthrough force as an assisting feature to enhance the resolution of AFM images. More recently, the robustness in the identification of the material-dependent breakthrough event, which emerges as a real molecular fingerprint, has opened up new research avenues to explore the nanomechanical properties of membranes of various compositions. However, this research field is probably still in its infancy, and many experimental points required further exploration. For example, it is still partially unclear the role of the supporting surface on the bilayer mechanical stability. Moreover, the chemistry of the AFM probe seems to have a strong effect on the measured mechanical response. Finally, the piezoelectric velocity and the temperature and composition of the liquid environment have also a crucial effect on the measured mechanical stability. Therefore, for the sake of comparison, experiments have to be conducted in a reproducible experimental set-up.

Setting these experimental concerns aside, applying force spectroscopy on lipid bilayers hold the promise to allow investigation of a number of scientific questions that can be now addressed at the nanometer scale. For example, the majority of works presented here are conducted on model lipid membranes, such as DPPC or DOTAP. Expanding these studies to other chemically distinct phospholipids will provide a new vista on the molecular determinants that confer mechanical stability to a membrane. Indeed, Garcia-Manyes et al. have recently studied the effect of the phospholipid chain length on the nanomechanics of lipid bilayers by comparing the force required to penetrate the bilayer for similar PC phospholipids that varied the number of $-CH_2-$ moieties in their hydrophobic tail [82]. This is shown in Fig. 6, where the distributions of breakthrough forces for PC phospholipids of varying lengths are shown. These results conclude

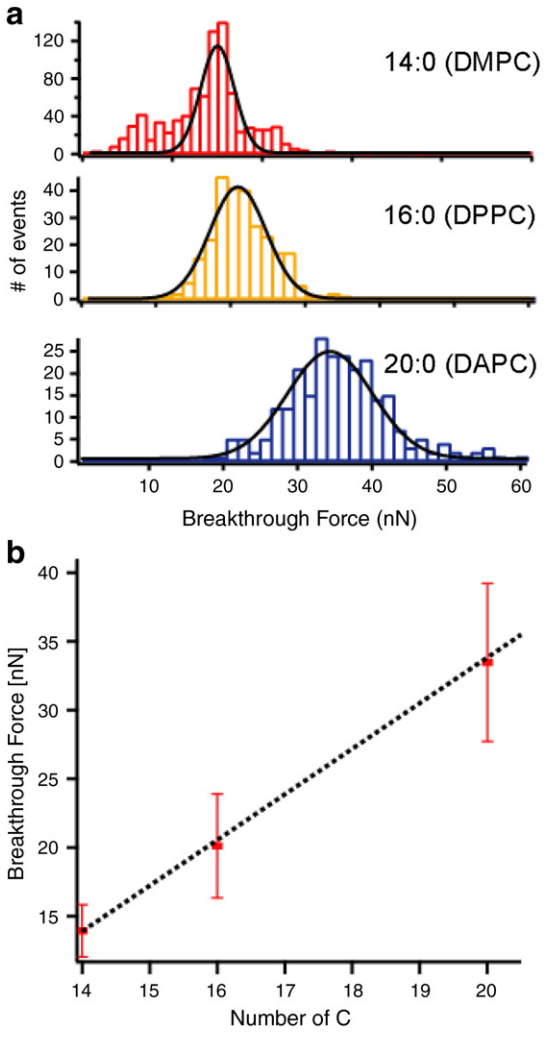

Fig. 6. The mechanical resistance of the lipid bilayer is highly dependent on the length of the phospholipid tail. (a) Distribution of breakthrough forces for the probed phosphatidylcholine phospholipids with varying lengths. Gaussian fits to the data yield a mean rupture force of 13.95 ± 1.87 nN, $n = 889$, DMPC (red); 19.66 ± 3.78 nN, $n = 266$, DPPC (orange); 33.5 ± 5.7 nN, $n = 263$, DAPC (blue). (b) Plot of the measured mean breakthrough force as a function of the number of carbons present in each phospholipid chain [82].

that the longer the apolar chain, the higher the force required to penetrate the membrane, probably due to enhanced van der Waals interactions. Whether the chemistry of the phospholipid headgroups has also an effect on the mechanical stability of the membranes remains currently elusive. A different powerful application of the determination of the breakthrough force is demonstrated in the experiments described above, where mixtures of different phospholipids could be identified in light of their different mechanical stability, even when they are forming domains of a only few nanometers. These experiments can be now extended to mixtures of phospholipids with enhanced complexity. The ultimate goal of the experimental approach reviewed here is certainly to fingerprint the mechanical stability of a full cellular membrane, which is composed of a complex mixture of phospholipids, membrane proteins and glycans in a wide range of compositions. However, we are still far from achieving such ambitious objective. The first step towards this long-term, challenging goal is to elucidate the role of each individual phospholipid on the overall mechanical stability of the membrane, requiring as a first approach model synthetic bilayers. Furthermore, since most of these bilayers have been used as templates for crystallization, a potential application for these localized nanomechanical tests would be probing whether the crystallization process has helped rigidify the bilayer itself.

Additionally, the nanomechanical experiments on lipid bilayers reviewed here permit a direct test of the role of different proteins and drugs on the mechanical properties of the membrane. Several pioneering studies have already shown the feasibility of the aforementioned experiments. For example, Mueller and coworkers, showed that the myelin basic protein (MBP) adopts a different conformation when in contact with a lipid bilayer [75,83]. Likewise, the bilayer is strongly modified by MBP attachment, indicating formation of MBP–lipid complexes and possibly disruption of the original bilayer structure. A similar case is observed for cytochrome *c*, which adsorbs to the membrane through electrostatic interactions [75]. A recent interesting report by Garcia-Saez et al. investigates the effect of Bax protein, a critical regulator of physiological cell death, on the nanomechanics of lipid bilayers [69]. In their study, the authors concluded that the force required to punch through a lipid bilayer in the presence of Bax decreased, in agreement with a two-state model of pore formation. Fitting their results to the continuum nucleation model proposed by Butt [33] yielded a reduction of a 30% in the pore line tension in the presence of the peptide. The combination of the highly localized AFM mechanical tests of the membrane reported here with other complementary approaches such as fluorescence (using confocal fluorescence imaging, FCS or two-focus FCS) offers an integrated picture able to provide structural and dynamical information on the membrane. For example, a recent report [60] has beautifully shown that the combination of both AFM and fluorescence techniques successfully characterizes the lipid–lipid and lipid–protein interactions governing the lateral organization of the membrane components.

Due to the nanometer-scale nature of our experiments, the experimental nanomechanical approach described here provides the ideal platform to bridge the gap with MD simulations, which uncover a variety of molecular mechanisms that are not accessible with mesoscopic experimental techniques [84,85]. Finally, from the technological viewpoint, these experiments will help in the design of tailored nano-devices that rely on the robustness of supported lipid bilayers [86]. To sum up, the results summarized here demonstrate the compelling effects of applying a highly localized mechanical force on lipid bilayers, a mechanism of common occurrence in nature.

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References

[1] W. Dowhan, Molecular basis for membrane phospholipid diversity: why are there so many lipids? Annu. Rev. Biochem. 66 (1997) 199–232.
[2] G. van Meer, D.R. Voelker, G.W. Feigenson, Membrane lipids: where they are and how they behave, Nat. Rev. Mol. Cell Biol. 9 (2008) 112–124.
[3] V. Vogel, M. Sheetz, Local force and geometry sensing regulate cell functions, Nat. Rev. Mol. Cell Biol. 7 (2006) 265–275.
[4] R. Phillips, T. Ursell, P. Wiggins, P. Sens, Emerging roles for lipids in shaping membrane-protein function, Nature 459 (2009) 379–385.
[5] D. Schmidt, Q.X. Jiang, R. MacKinnon, Phospholipids and the origin of cationic gating charges in voltage sensors, Nature 444 (2006) 775–779.
[6] D. Schmidt, R. MacKinnon, Voltage-dependent K+ channel gating and voltage sensor toxin sensitivity depend on the mechanical state of the lipid membrane, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 19276–19281.
[7] D. Oliver, C.C. Lien, M. Soom, T. Baukrowitz, P. Jonas, B. Fakler, Functional conversion between A-type and delayed rectifier K+ channels by membrane lipids, Science 304 (2004) 265–270.
[8] Y. Ramu, Y. Xu, Z. Lu, Enzymatic activation of voltage-gated potassium channels, Nature 442 (2006) 696–699.
[9] E. Perozo, A. Kloda, D.M. Cortes, B. Martinac, Physical principles underlying the transduction of bilayer deformation forces during mechanosensitive channel gating, Nat. Struct. Biol. 9 (2002) 696–703.
[10] T.M. Suchyna, S.E. Tape, R.E. Koeppe II, O.S. Andersen, F. Sachs, P.A. Gottlieb, Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers, Nature 430 (2004) 235–240.
[11] H. Hong, L.K. Tamm, Elastic coupling of integral membrane protein stability to lipid bilayer forces, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4065–4070.
[12] B.J. Reynwar, G. Illya, V.A. Harmandaris, M.M. Muller, K. Kremer, M. Deserno, Aggregation and vesiculation of membrane proteins by curvature-mediated interactions, Nature 447 (2007) 461–464.
[13] H.J. Lee, E.L. Peterson, R. Phillips, W.S. Klug, P.A. Wiggins, Membrane shape as a reporter for applied forces, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 19253–19257.
[14] C.A. Rutkowski, L.M. Williams, T.H. Haines, H.Z. Cummins, The elasticity of synthetic phospholipid-vesicles obtained by photon-correlation spectroscopy, Biochemistry 30 (1991) 5688–5696.
[15] F.R. Hallett, J. Marsh, B.G. Nickel, J.M. Wood, Mechanical-properties of vesicles. 2. A model for osmotic swelling and lysis, Biophys. J. 64 (1993) 435–442.
[16] B.L.S. Mui, P.R. Cullis, E.A. Evans, T.D. Madden, Osmotic properties of large unilamellar vesicles prepared by extrusion, Biophys. J. 64 (1993) 443–453.
[17] W. Rawicz, K.C. Olbrich, T. McIntosh, D. Needham, E. Evans, Effect of chain length and unsaturation on elasticity of lipid bilayers, Biophys. J. 79 (2000) 328–339.
[18] E. Evans, W. Rawicz, Entropy-driven tension and bending elasticity in condensed-fluid membranes, Phys. Rev. Lett. 64 (1990) 2094–2097.
[19] V. Heinrich, W. Rawicz, Automated, high-resolution micropipet aspiration reveals new insight into the physical properties of fluid membranes, Langmuir 21 (2005) 1962–1971.
[20] E. Evans, V. Heinrich, F. Ludwig, W. Rawicz, Dynamic tension spectroscopy and strength of biomembranes, Biophys. J. 85 (2003) 2342–2350.
[21] J. Marra, J. Israelachvili, Direct measurements of forces between phosphatidylcholine and phosphatidylethanolamine bilayers in aqueous electrolyte solutions, Biochemistry 24 (1985) 4608–4618.
[22] J. Marra, Direct measurement of the interaction between phosphatidylglycerol bilayers in aqueous electrolyte solutions, Biophys. J. 50 (1986) 815–825.
[23] C.A. Helm, J.N. Israelachvili, P.M. McGuiggan, Molecular mechanisms and forces involved in the adhesion and fusion of amphiphilic bilayers, Science 246 (1989) 919–922.
[24] M. Benz, T. Gutsmann, N.H. Chen, R. Tadmor, J. Israelachvili, Correlation of AFM and SFA measurements concerning the stability of supported lipid bilayers, Biophys. J. 86 (2004) 870–879.
[25] B. Seantier, M.C. Giocondi, C. Le Grimellec, P.E. Milhiet, Probing supported model and native membranes using AFM, Curr. Opin. Colloid Interface Sci. 13 (2008) 326–337.
[26] M.P. Mingeot-Leclercq, M. Deleu, R. Brasseur, Y.F. Dufrene, Atomic force microscopy of supported lipid bilayers, Nat. Protoc. 3 (2008) 1654–1659.
[27] E.I. Goksu, J.M. Vanegas, C.D. Blanchette, W.C. Lin, M.L. Longo, AFM for structure and dynamics of biomembranes, Biochim. Biophys. Acta 1788 (2009) 254–266.
[28] B. Seantier, C. Breffa, O. Felix, G. Decher, Dissipation-enhanced quartz crystal microbalance studies on the experimental parameters controlling the formation of supported lipid bilayers, J. Phys. Chem. B 109 (2005) 21755–21765.
[29] A. Slade, J. Luh, S. Ho, C.M. Yip, Single molecule imaging of supported planar lipid bilayer—reconstituted human insulin receptors by in situ scanning probe microscopy, J. Struct. Biol. 137 (2002) 283–291.
[30] J. Schneider, W. Barger, G.U. Lee, Nanometer scale surface properties of supported lipid bilayers measured with hydrophobic and hydrophilic atomic force microscope probes, Langmuir 19 (2003) 1899–1907.
[31] Y.F. Dufrene, T. Boland, J.W. Schneider, W.R. Barger, G.U. Lee, Characterization of the physical properties of model biomembranes at the nanometer scale with the atomic force microscope, Faraday Discuss. (1998) 79–94 (discussion 137–157).
[32] Y.F. Dufrene, W.R. Barger, J.B.D. Green, G.U. Lee, Nanometer-scale surface properties of mixed phospholipid monolayers and bilayers, Langmuir 13 (1997) 4779–4784.

[33] H.J. Butt, V. Franz, Rupture of molecular thin films observed in atomic force microscopy. I. Theory, Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 66 (2002) 031601.
[34] S. Loi, G. Sun, V. Franz, H.J. Butt, Rupture of molecular thin films observed in atomic force microscopy. II. Experiment, Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 66 (2002) 031602.
[35] V. Franz, S. Loi, H. Muller, E. Bamberg, H.H. Butt, Tip penetration through lipid bilayers in atomic force microscopy, Colloids Surf. B-Biointerfaces 23 (2002) 191–200.
[36] S. Kunneke, D. Kruger, A. Janshoff, Scrutiny of the failure of lipid membranes as a function of headgroups, chain length, and lamellarity measured by scanning force microscopy, Biophys. J. 86 (2004) 1545–1553.
[37] S.G. Corcoran, R.J. Colton, E.T. Lilleodden, W.W. Gerberich, Anomalous plastic deformation at surfaces: nanoindentation of gold single crystals, Phys. Rev. B 55 (1997) 16057–16060.
[38] M. Carrion-Vazquez, A.F. Oberhauser, S.B. Fowler, P.E. Marszalek, S.E. Broedel, J. Clarke, J.M. Fernandez, Mechanical and chemical unfolding of a single protein: a comparison, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 3694–3699.
[39] P.E. Marszalek, H. Li, A.F. Oberhauser, J.M. Fernandez, Chair-boat transitions in single polysaccharide molecules observed with force-ramp AFM, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 4278–4283.
[40] H. Clausen-Schaumann, M. Rief, C. Tolksdorf, H.E. Gaub, Mechanical stability of single DNA molecules, Biophys. J. 78 (2000) 1997–2007.
[41] J. Fraxedas, S. Garcia-Manyes, P. Gorostiza, F. Sanz, Nanoindentation: toward the sensing of atomic interactions, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5228–5232.
[42] S. Garcia-Manyes, L. Dougan, C.L. Badilla, J. Brujic, J.M. Fernandez, Direct observation of an ensemble of stable collapsed states in the mechanical folding of ubiquitin, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 10534–10539.
[43] E.L. Florin, M. Rief, H. Lehmann, M. Ludwig, C. Dornmair, V.T. Moy, H.E. Gaub, Sensing specific molecular-interactions with the atomic-force microscope, Biosens. Bioelectron. 10 (1995) 895–901.
[44] W.A. Ducker, D.R. Clarke, Controlled modification of silicon–nitride interactions in water via zwitterionic surfactant adsorption, Colloids Surf. a-Physicochemical and Engineering Aspects 93 (1994) 275–292.
[45] J. Torrent-Burgues, G. Oncins, F. Sanz, Study of mixed Langmuir and Langmuir–Blodgett films of dissimilar components by AFM and force spectroscopy, Colloids Surf. a-Physicochemical and Engineering Aspects 321 (2008) 70–75.
[46] G. Oncins, J. Torrent-Burgues, F. Sanz, Nanomechanical properties of arachidic acid Langmuir–Blodgett films, J. Phys. Chem. C 112 (2008) 1967–1974.
[47] S. Garcia-Manyes, O. Domenech, F. Sanz, M.T. Montero, J. Hernandez-Borrell, Atomic force microscopy and force spectroscopy study of Langmuir–Blodgett films formed by heteroacid phospholipids of biological interest, Biochim. Biophys. Acta 1768 (2007) 1190–1198.
[48] L.T.W. Lim, A.T.S. Wee, S.J. O'Shea, Temperature dependence of solvation forces as measured in atomic force microscopy, J. Chem. Phys. 130 (2009).
[49] S.J. Oshea, M.E. Welland, T. Rayment, Solvation forces near a graphite surface measured with an atomic force microscope, Appl. Phys. Lett. 60 (1992) 2356–2358.
[50] S.J. O'Shea, M.E. Welland, Atomic force microscopy at solid–liquid interfaces, Langmuir 14 (1998) 4186–4197.
[51] G.X. Sun, E. Bonaccurso, V. Franz, H.J. Butt, Confined liquid: simultaneous observation of a molecularly layered structure and hydrodynamic slip, J. Chem. Phys. 117 (2002) 10311–10314.
[52] N.N. Gosvami, S.K. Sinha, S.J. O'Shea, Squeeze-out of branched alkanes on graphite, Phys. Rev. Lett. 100 (2008).
[53] N.N. Gosvami, S.K. Sinha, W. Hofbauer, S.J. O'Shea, Solvation and squeeze out of hexadecane on graphite, J. Chem. Phys. 126 (2007) 214708.
[54] B. Cappella, G. Dietler, Force-distance curves by atomic force microscopy. Surface Science Reports 34 (1999) 1–+.
[55] S. Garcia-Manyes, G. Oncins, F. Sanz, Effect of ion-binding and chemical phospholipid structure on the nanomechanics of lipid bilayers studied by force spectroscopy, Biophys. J. 89 (2005) 1812–1826.
[56] S. Garcia-Manyes, G. Oncins, F. Sanz, Effect of temperature on the nanomechanics of lipid bilayers studied by force spectroscopy, Biophys. J. 89 (2005) 4261–4274.
[57] Z.V. Leonenko, E. Finot, H. Ma, T.E. Dahms, D.T. Cramb, Investigation of temperature-induced phase transitions in DOPC and DPPC phospholipid bilayers using temperature-controlled scanning force microscopy, Biophys. J. 86 (2004) 3783–3793.
[58] M. Li, M. Chen, E. Sheepwash, C.L. Brosseau, H. Li, B. Pettinger, H. Gruler, J. Lipkowski, AFM studies of solid-supported lipid bilayers formed at a Au(111) electrode surface using vesicle fusion and a combination of Langmuir–Blodgett and Langmuir–Schaefer techniques, Langmuir 24 (2008) 10313–10323.
[59] M.H. Chen, M. Li, C.L. Brosseau, J. Lipkowski, AFM studies of the effect of temperature and electric field on the structure of a DMPC-cholesterol bilayer supported on a Au(111) electrode surface, Langmuir 25 (2009) 1028–1037.
[60] S. Chiantia, J. Ries, N. Kahya, P. Schwille, Combined AFM and two-focus SFCS study of raft-exhibiting model membranes, Chemphyschem 7 (2006) 2409–2418.
[61] J. Schneider, Y.F. Dufrene, W.R. Barger Jr., G.U. Lee, Atomic force microscope image contrast mechanisms on supported lipid bilayers, Biophys. J. 79 (2000) 1107–1118.
[62] M.R. Nussio, G. Oncins, I. Ridelis, E. Szili, J.G. Shapter, F. Sanz, N.H. Voelcker, Nanomechanical characterization of phospholipid bilayer islands on flat and porous substrates: a force spectroscopy study, J. Phys. Chem. B 113 (2009) 10339–10347.
[63] S. Garcia-Manyes, G. Oncins, F. Sanz, Effect of pH and ionic strength on phospholipid nanomechanics and on deposition process onto hydrophilic surfaces measured by AFM, Electrochim. Acta 51 (2006) 5029–5036.
[64] R.P. Richter, A. Brisson, Characterization of lipid bilayers and protein assemblies supported on rough surfaces by atomic force microscopy, Langmuir 19 (2003) 1632–1640.
[65] I. Pera, R. Stark, M. Kappl, H.J. Butt, F. Benfenati, Using the atomic force microscope to study the interaction between two solid supported lipid bilayers and the influence of synapsin I, Biophys. J. 87 (2004) 2446–2455.
[66] H.J. Butt, Electrostatic interaction in atomic force microscopy, Biophys. J. 60 (1991) 777–785.
[67] S. Garcia-Manyes, P. Gorostiza, F. Sanz, Titration force microscopy on supported lipid bilayers, Anal. Chem. 78 (2006) 61–70.
[68] J. Israelachvili, Intermolecular and Surface Forces, Academic Press, London, 1991.
[69] A.J. Garcia-Saez, S. Chiantia, J. Salgado, P. Schwille, Pore formation by a bax-derived peptide: effect on the line tension of the membrane probed by AFM, Biophys. J. 93 (2007) 103–112.
[70] L.M. Grant, F. Tiberg, Normal and lateral forces between lipid covered solids in solution: correlation with layer packing and structure, Biophys. J. 82 (2002) 1373–1385.
[71] A.M. Trunfio-Sfarghiu, Y. Berthier, M.H. Meurisse, J.P. Rieu, Role of nanomechanical properties in the tribological performance of phospholipid biomimetic surfaces, Langmuir 24 (2008) 8765–8771.
[72] G. Oncins, S. Garcia-Manyes, F. Sanz, Study of frictional properties of a phospholipid bilayer in a liquid environment with lateral force microscopy as a function of NaCl concentration, Langmuir 21 (2005) 7373–7379.
[73] R.A. Bockmann, H. Grubmuller, Multistep binding of divalent cations to phospholipid bilayers: a molecular dynamics study, Angew. Chem. Int. Ed. Engl. 43 (2004) 1021–1024.
[74] R.A. Bockmann, A. Hac, T. Heimburg, H. Grubmuller, Effect of sodium chloride on a lipid bilayer, Biophys. J. 85 (2003) 1647–1655.
[75] H. Mueller, H.J. Butt, E. Bamberg, Adsorption of membrane-associated proteins to lipid bilayers studied with an atomic force microscope: myelin basic protein and cytochrome *c*, J. Phys. Chem. B 104 (2000) 4552–4559.
[76] T. Heimburg, A.D. Jackson, On soliton propagation in biomembranes and nerves, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9790–9795.
[77] D. Keller, N.B. Larsen, I.M. Moller, O.G. Mouritsen, Decoupled phase transitions and grain-boundary melting in supported phospholipid bilayers, Phys. Rev. Lett. 94 (2005) 025701.
[78] G. Oncins, L. Picas, J. Hernandez-Borrell, S. Garcia-Manyes, F. Sanz, Thermal response of Langmuir–Blodgett films of dipalmitoylphosphatidylcholine studied by atomic force microscopy and force spectroscopy, Biophys. J. 93 (2007) 2713–2725.
[79] L. Picas, M.T. Montero, A. Morros, G. Oncins, J. Hernandez-Borrell, Phase changes in supported planar bilayers of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine, J. Phys. Chem. B 112 (2008) 10181–10187.
[80] R.M. Sullan, J.K. Li, S. Zou, Direct correlation of structures and nanomechanical properties of multicomponent lipid bilayers, Langmuir 25 (13) (2009) 7471–7477.
[81] L. Picas, M.T. Montero, A. Morros, M.E. Cabanas, B. Seantier, P.E. Milhiet, J. Hernandez-Borrell, Calcium-induced formation of subdomains in phosphatidylethanolamine–phosphatidylglycerol bilayers: a combined DSC, P-31 NMR, and AFM study, J. Phys. Chem. B 113 (2009) 4648–4655.
[82] S. Garcia-Manyes, G. Oncins, L. Redondo, F. Sanz, Nanomechanics of lipid bilayers: heads or tails? Biophys. J. 98 (3) (2010) 75a.
[83] H. Mueller, H.J. Butt, E. Bamberg, Force measurements on myelin basic protein adsorbed to mica and lipid bilayer surfaces done with the atomic force microscope, Biophys. J. 76 (1999) 1072–1079.
[84] T. Rog, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen, Ordering effects of cholesterol and its analogues, Biochim. Biophys. Acta 1788 (2009) 97–121.
[85] M.L. Berkowitz, Detailed molecular dynamics simulations of model biological membranes containing cholesterol, Biochim. Biophys. Acta 1788 (2009) 86–96.
[86] A. Meister, M. Gabi, P. Behr, P. Studer, J. Voros, P. Niedermann, J. Bitterli, J. Polesel-Maris, M. Liley, H. Heinzelmann, T. Zambelli, FluidFM: combining atomic force microscopy and nanofluidics in a universal liquid delivery system for single cell applications and beyond, Nano Lett. 9 (2009) 2501–2507.