Supplementary Material (ESI) for Soft Matter

Nanoscale Structure of Lipid Domain Boundaries

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Some basic insights into force measurements with AFM are provided here to help the reader understand the measurements discussed in the manuscript.

Force Spectroscopy

The ability to independently control the AFM cantilever displacement normal to the sample surface enables a technique known as force spectroscopy to be utilised. This technique monitors the cantilever deflection (d) as a function of vertical displacement (z) of the piezoelectric scanner. As raw data yields a cantilever deflection vs. scanner displacement curve, the data can be transformed into a force(F) vs. distance(D) curve with the aid of Hooke's law (Eq. 1) to convert the cantilever deflection(d) into a measurement of force(F).[1, 2]

$$F = -k\Delta d \qquad (1)$$

where k is the cantilever spring constant, and $\Delta D = \Delta z - \Delta d$ is the probe-sample separation.

It is important to calibrate the spring constant, as actual spring constant values may differ substantially from those quoted by the manufacturer. Common techniques for the calibration of individual spring constants use either a dynamic method, reliant on the knowledge of mass and resonance frequency of the tip[3] or an equipartition theorem,[4] that requires the tip piezo sensitivity to measure an accurate value of spring constant by the use of thermal noise.

As there are various features in a force curve that provide information on surface properties and molecular interactions, force spectroscopy has emerged as a valuable tool for the surface characterisation of biomolecules[5-8] and cell research.[9-14] A schematic of a typical force curve is shown in Figure 1. At large tip-sample separation distances, there is no deflection experienced by the cantilever and hence the interactions forces measured by the tip are zero (Fig. 1A). The tip is then moved closer to the sample surface, potentially bending upward as a result of repulsive forces, such as electrostatic,[15, 16] hydration[15, 17] and steric forces.[18] Approaching further, the tip is pulled down to the sample surface by attractive forces, as the gradient of attractive forces exceeds the spring constant in addition to the gradient of

repulsive forces (Fig. 1B).[19] The approach portion of the force curve is particularly useful for the measurement of van der Waals, electrostatic, solvation, hydration and steric/bridging forces.[2] Any additional movement causes the tip to press into the sample surface, which is characterised by an increase in deflection as the cantilever is bending upward (Fig. 1C). Dependant on the sample stiffness, different behaviours can be observed in the contact region of a force curve. In particularly, on hard non-deformable surfaces, a typical vertical line representative of increasing deflection is observed (as seen in Fig. 1C). However, in the case of soft samples, an indentation will occur and this is characterised by a discontinuity in the contact region of the force curve. This typical feature of force spectroscopy can be used to yield information on film thickness and mechanical properties of soft layers.[20-22] It has been demonstrated as a useful tool to study the nanomechanical properties of lipid bilayers[7, 8, 23, 24] and adsorbed surfactant films.[25, 26]

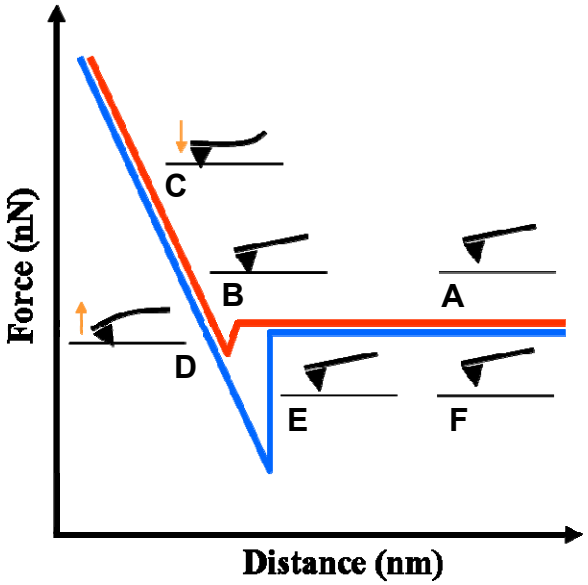

Figure 1. Schematic of a force-distance curve, the red and blue lines are indicated of the approach and retraction cycles of the AFM tip to the substrate surface, respectively. Adapted from references 2, 27.

Retracting the tip from the sample surface allows the cantilever to relax downward (Fig. 1D) until the tip forces are in equilibrium with the surface forces. Further retraction can often exhibit a hysteresis referred to as the adhesion 'pull off force' (Fig. 1E). The maximum adhesion is referred to as the point where the tip finally breaks free from the surface attraction and the forces acting on the tip return to zero (Fig. 1F). Adhesion forces have been demonstrated to be particularly useful in field of molecular recognition, being capable of measuring the unbinding forces between complementary biomolecules, and the surface energy of solids.[20, 28, 29]

In this work, force plots were acquired using either triangular Si_3N_4 cantilevers or modified Si_3N_4 cantilevers with a nominal spring constant of 0.08 N m^{-1}, 0.15 N m^{-1} or 0.12 N m^{-1}. As the dimensions of cantilevers can vary significantly, spring constants were calculated for unmodified and modified probes by the method developed by Sader et al.[3] or using the equipartition theorem (thermal noise)[4] after determination of the tip sensitivity (Vnm^{-1}). Vertical spring constant force calibration using the equipartition theorem was performed with a force probe 1D MFP (Asylum Research, Santa Barbara, CA). The measured force constants

agreed with the manufacturer's nominal values for both the unmodified and modified probes. The quality factor (Q-factor) of the cantilever for unmodified and modified probes yielded Q ~ 61 and 58, respectively. All force curve measurements acquired 256 sample data points.

Force Spectroscopy coupled with Topography Measurements

A variety of approaches that can map recognition forces with the same resolution of the simultaneously collected topographic images been reported.[30-34] Utilising pulsed force mode scanning force microscopy (PFM-SFM), contrasts in adhesion for ternary mixtures of SPBs have been measured.[30] Higher adhesion was observed for 1,2-diolely-phosphatidylcholine (DOPC) rich phases, in contrast to domains rich in sphingomyelin and cholesterol. The adhesion contrast was a result of the tip penetrating deeper into the DOPC liquid phase, thus providing a larger contact area with the tip. No attempt was made in this study to use defined tip surface chemistry in order to differentiate between individual membrane components. The so called Topopgraphy and Recognition (TREC) imaging relies on functionalistion of the AFM tip with a species that will interact specifically with a surface species.[32-34] TREC type imaging has focused on imaging location of proteins or receptors within membranes and cell surfaces but has not been used extensively to image the membranes themselves.[32, 34, 35] These techniques have the ability to provide high spatial resolution images of membrane bilayers but do not provide post-processing quantitative force curves as only the stiffness (slope) and adhesion (pull-off force) from each point, which are then used to make the images of each property, are stored.

A recent development in measuring force curves is Digital Pulsed Force Mode (DPFM). The extension in this approach is that force curves at each point can be stored and analysed. However, the resolution of the acquired force curves is very low. The force applied to the surface in these pulsed modes is somewhat difficult to control and hence surface damage is a possibility as well.[36] This is a severe limitation for soft samples such as SLBs because they are very easy to penetrate and such disruption of the layer will certainly yield incorrect results for both topography and forces. Additionally such penetration will likely destroy the layer and contaminate the AFM probes.

Force Volume Imaging Mode

In combination with force spectroscopy and contact-mode imaging, force-volume (FV) mode has become a popular approach to simultaneously map surface topography and surface properties. With the aid of chemically functionalised tips, chemical mapping has also become a viable option for the analysis of cell surfaces using FV imaging.[37] In fact, the first proof of concept of this 'affinity imaging mode' was performed using biotinylated functionalised tips, whilst imaging microscale streptavidin patterns.[38] Chemical functionalisation of AFM probes adds the selective sensing capabilities of certain interactions. FV imaging collects force curves at each x,y pixel of the image by measuring cantilever deflection. Whilst a height image is also produced by this technique, the strength of this technique is that the information contained in the 3D data set can be decoupled from the topographic information. The disadvantage, however, is the long time required to acquire a single data set. The time resolution of this technique currently requires between 2-30 mins, dependant on the acquisition parameters. Unfortunately dynamic processes in biology usually

occur at much smaller time scales than those measurable with this technique, however, the improvement of scientific instrumentation will see FV become a valuable tool in cell surface research.

In this work, FV images (32 x 32 or 64 x 64 force curves) were collected at a scan rate of 0.0723Hz-0.0962Hz, in relative trigger mode. An appropriate trigger mechanism was set for cantilever deflection. All force measurements presented are given with ± one standard deviation.

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