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Imaging of Mechanical Properties of Soft Matter

From Heterogeneous Polymer Surfaces to Single Biomolecules

In recent years the **atomic force microscope** (AFM) has evolved from a high resolution imaging tool to an enabling platform for physical studies at the nanoscale including quantitative mapping of mechanical characteristics of surfaces providing simultaneous topography and mechanical property maps across the length scales. In the work presented here **peak force tapping AFM** was utilized to elaborate the nanoscale mechanical performance of phase separated polyurethanes (PUs) and the mechanical properties of lysozyme molecules adsorbed to mica substrates.

Introduction

Atomic force microscopy (AFM) has become a true enabling platform in soft matter science, and specifically in macromolecular nanotechnology, including biological systems. Despite the tremendous progress in AFM technology development it has remained notoriously difficult to obtain quantitative mechanical maps of the elastic performance of soft matter with high resolution. Tapping mode imaging was a pivotal development in AFM technology and became a routinely used imaging mode to study polymer surfaces, allowing gentle scanning with significantly reduced lateral forces [1,2]. However, it has not allowed one to obtain quantitative mechanical maps because the phase signal is related to the energy dissipation of the tapping tip [3]. There is an ongoing effort in academic research and industrial instrumental development aiming at improved or fundamentally new imaging modes enabling quantitative high resolution mechanical imaging by AFM.

Since our last report [2] remarkable achievements have been made in AFM imaging technology and quantitative mechanical maps can now be obtained simultaneously with topography imaging exhibiting resolution all the way down to the nanoscale. In general, three main approaches can be distinguished, including multifrequency tapping, pulsed force techniques [3,4,5] and recently introduced contact resonance techniques [6] which operate in contact mode with dynamic excitation at or near the cantilever resonance. They seem particularly promising for the study of viscoelastic materials providing loss and storage modulus maps of a material surface.

To date these approaches are being continuously developed and commercialization is also underway by different manufactures.

Peak force tapping AFM has been introduced as an AFM based mechanical property mapping technique [7]. In a peak force tapping experiment the sample is oscillated at a rate well below the resonance frequency of the AFM cantilever. The AFM feedback uses the maximum force load (peak force) as its control signal to maintain a constant imaging force. As a result multiple force vs. time curves are being recorded and averaged on each probed sample pixel. From the corresponding force distance curves mechanical parameters like adhesion, deformation and the elastic modulus are determined as illustrated in figure 1.

In order to estimate the elastic performance from the force distance curves the Derjaguin-Müller-Toporov (DMT) mechanical contact model was used in our experiments. According to the DMT model the forces of the AFM tip-surface interaction are: **see figure 5** where $F_{interaction}$ is the tip-sample-force, E* is the reduced elastic modulus, *r* is the contact radius of the AFM tip, d_0 is the surface rest position, $(d\text{-}d_0)$ is the deformation of the sample and F_{adh} is the adhesion force. The DMT model was found to be useful for samples with moderate adhesion levels and AFM tips with small radii. Importantly, it allows feasible computation, rendering it favorable for real time imaging. It is important to mention that independent of the chosen contact mechanical model the AFM tip geometry, its penetration depth and hence the contact area as expressed in the contact radius *r* do have a pronounced effect on the values of the moduli obtained. This is corrected to some extent by using a calibration procedure with a microphase separated polymer of known modulus. In the measurements the same setpoints are chosen as for the calibration [7]. Still local topography or roughness can have an impact on the measured moduli values due to variation in contact radii. Consequently flat samples with minimal roughness represent optimal specimen for the mechanical mapping.

Mechanical Mapping of Phase Separated Polyurethanes

The quantitative characterization of the morphology and mechanical performance of microphase separated polymeric structures is essential not only for a better understanding of material behavior, but also for the design and preparation of novel materials with well-defined end properties. Polyurethanes (PUs) represent a versatile class of soft matter with a wide variety of specific applications. PUs can be designed to possess constituents of either soft or stiff mechanical characteristics with the proper control of their molecular structure to render it useful for the particular end-use targeted. A typical linear segmented PU elastomer usually consists of an aromatic diisocyanate, in our case diphenylmethane-4-4'-diisocyanate (MDI), butanediol chain extender and a polyester or polyether polyol. The segments can phase separate in soft and hard phases in a complex fashion. Although the amount of soft and hard segments can in principle be determined from the composition of the reaction mixture, the composition of the phases is a priori unknown. Moreover, segmented polyurethanes were shown to form hierarchical structures with several structural units at different length scales [8].

We investigated segmented polyether polyurethanes exhibiting a variation of the stoichiometric ratios of the isocyanate (NCO) and the hydroxyl (OH) groups [7]. To obtain sufficiently flat surfaces the PU samples were cryomicrotomed before AFM imaging. Quantitative DMT elastic modulus and adhesion maps were obtained simultaneously (fig. 2). The color code in figure 2 corresponds to variations in the values of elastic moduli and in adhesion forces, respectively, both showing a large mechanical heterogeneity of the PU surface (sample A). The elastic moduli of hard (bright color) and soft (dark color) segments of the phase separated polyurethane samples are mapped with nanoscale resolution (fig. 2A). The sample reveals a wavy morphology of the hard segments assembled into larger domains that extend over few 100 nm^2 areas. The observed elastic modulus values ranged from ~22-150 MPa while adhesive forces revealed values ranging from ~1.6-4.2 nN.

The comparison of the elastic modulus maps of two different PU samples prepared with different NCO/OH ratios (sample A: 0.94 (same as in fig. 2); sample B: 1.025) is shown in figure 3. Strikingly, both samples reveal a significantly different morphology. In contrast to the already described sample A (fig. 2), the hard segments of sample B show sharp features of straight elongated features with random orientation. Figure 3 includes a detailed analysis of the respective surface elastic moduli with cross-sections and modulus distributions over the entire scanned areas. The measured DMT modulus values show a wide distribution for both PU samples, and range between ~25 150 MPa for sample A, and ~60 210 MPa

for sample B. Mean values derived from the DMT modulus histograms (elastic modulus value at histogram maximum) are 44.8 MPa (sample A) and 78.5 MPa (sample B), respectively. The AFM derived surface mean modulus values do not coincide with the bulk values obtained by tensile testing of 65±5 MPa (sample A) and 25±5 MPa (sample B) [7].

Firstly, we attribute this difference to fundamentally different averaging procedures and effects. Tensile moduli are determined by the physical averaging process of the tensile experiment and the corresponding bulk tensile modulus values depend on volume and not on surface fractions. Importantly, the PU samples possess a complex morphology, and each composition has its own structure and modulus, which is probably not in thermodynamic equilibrium after tensile testing. In addition the apparent modulus depends on orientation, and thermo-rheological history of the specific sample. In contrast, AFM based mechanical mappings give a very detailed insight into the nanoscopic morphology with a broad distribution of surface moduli of the PUs at a surface exposed following microtoming. If one considers a two phase system of the PU featuring stiff and soft segments, the mean surface modulus should depend only on the relative surface area of each phase, provided that the elastic modulus values for the constituents remain the same. In our case the PU samples exhibit domains and topographical features that are in the regime of tens of nanometers. Consequently confinement effects of the soft and hard segments mutually influencing each other might broaden the distribution of modulus values. Moreover, at the boundaries between the soft and hard segments an averaging of moduli will occur in the regime of the contact area of the AFM tip. The used AFM tips had very small nominal radii of 2-3 nm. Thus we assume that local averaging at boundaries is minimal. Moreover, these small tip radii also minimize potential modulus errors caused by local topography as increased roughness leads to a larger contact radius r, which directly reflects in the apparent elastic modulus. Significant tapping frequency dependencies were reported recently in lower frequency regimes (< ~600 Hz) on low density polyethylene (LDPE) with comparable bulk elastic moduli [9]. For the PU elastomers no substantial frequency dependences are anticipated in the applied tapping frequency (2 kHz).We found similar modulus values at higher probing frequencies (~50 kHz) utilizing a multifrequency approach (data not shown here) [7].

Mechanical Mapping of Enzymes

The study of mechanical properties of proteins such as deformability and flexibility is of fundamental importance since protein functions and their three-dimensional conformations are intimately connected. A few cases are reported in the literature where AFM has been used to compress protein molecules to extract information on

the apparent Young's moduli of single molecules or monolayers [10-15]. Strikingly, the denaturation process of a single protein could be detected in this manner [11]. Here we describe the mechanical properties of surface adsorbed lysozyme. Lysozyme is a globular protein with a molecular mass of 14.4 kDa. It damages bacterial cell walls by hydrolyzing β(1,4) glucosidic bonds in the peptidoglycan layer of bacteria. In our studies the enzyme was adsorbed from solution to a freshly cleaved mica surface due to electrostatic interaction. Mechanical maps of lysozyme monolayers were obtained in physiological buffered environment, both on (diluted) monolayers as well as single enzymes providing DMT modulus and deformation maps (fig. 4).

Adhesion differences were minimal (data not shown). The height values varied between ~2-3 nm and lateral dimensions were also in excellent agreement with the molecular dimensions. Deformations were recorded between 0.5-1 nm which indicates a partial compression of the molecules, assuring to not completely compress the molecule and thereby minimize also the influence of the underlying substrate. On the other hand, the molecule must be compressed to some extent to be able to determine elastic moduli. DMT modulus values obtained on the lysozyme samples showed mean values of ~140 200 MPa with an entire modulus value range from ~80-250 MPa. We found good agreement with literature values obtained by AFM force volume (FV) imaging in previous studies of 500±200 MPa obtained on lysozyme [10] and 600 ± 200 MPa on lactate oxidase [12]. However these modulus values are at least 1-2 orders of magnitude larger than the ones recorded on the native purple membrane (PM) from Halobacterium salinarum [13] which consists of the light-driven proton-pump bacteriorhodopsin (BR) and lipids. The Young's moduli of both PM surfaces revealed 10±5 MPa in one study [13]. These significantly lower modulus values may be due to the presence of the lipids which are softer than the protein and the 2D nature of the membrane. Lateral mobility within the membrane might lead to an apparent softening of the sample under gentle indentation forces. The possible contribution of an underlying hard substrate on the elastic properties of thin enzyme layers must be carefully elaborated and is currently under investigation, in addition to probing frequency dependencies. In this regard similar mechanical performance was found for bacteriorhodopsin at 2 kHz [14] and ~50 kHz [15] probing frequencies.

Conclusion

AFM has evolved from a high resolution imaging tool into a mechanical mapping technique providing topography and quantitative mechanical maps across the length scales simultaneously. The feasibility of peak force tapping AFM to obtain mechanical maps on phase separated polyurethane surfaces and on lysozyme

monolayers on mica has been demonstrated in this article.

Despite the remarkable progress in the development of the AFM based mechanical imaging technology including commercial availability, a number of physical aspects must be addressed in the future and taken into careful consideration: effects of surface roughness and frequency of probing, the choice of the appropriate contact mechanics model and the potential impact of the underlying hard substrate if thin films are investigated.

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