Lateral force-distance curve technique

D S Bulgarevich, K Mitsui and H Arakawa
Organic Nanomaterials Center, National Institute for Materials Science, 1-1, Namiki, Tsukuba 305-0044, Japan
ARAKAWA.Hideo@nims.go.jp

Abstract. Nano-mechanical properties of single biomolecules and polymers as well as interaction forces between specific molecular pairs have been intensively studied during the last two decades by using atomic force microscopy with force measurement technique. In these experiments, molecules were pulled up from the substrate in vertical direction. However, it is also interesting to develop a similar lateral force-distance technique in which sample molecules stay lain on the solid surfaces during mechanical stretching. Thus, wider possibilities can be opened up to study the stretched macromolecules by other microscopy/spectroscopy methods. Since typical atomic force microscopes have the lateral scanner ranges up to ~100 μm, very long macromolecules can be studied and manipulated in this way. As an example, up to 8 μm stretching of self-polymerized poly(ethylene glycol)-bis-thiol on gold surface was observed. It was also confirmed that even in the lateral force-distance curves (50-300 nm above surface), the vertical cantilever bending, not torsion, was mainly responsible for the observed deflection signal. This made lateral force data analysis simpler since the normal force constants are easier to estimate than torsion ones. The force curves in our method were also free of any interference fringes. They had horizontal and easy identifiable baselines.

1. Introduction
The first force-distance curve measurements and their interpretations in air and liquids go back to 1988-1991. Nowadays, it is a well-established technique of atomic force microscopy, which allows the various microscopic adhesion, meniscus, and hydrodynamic force studies, as well as the investigations of nano-mechanical properties of single biomolecules and polymers. There are several extensive reviews in literature on these topics [1-6]. In typical studies of single molecule stretching with atomic force microscope (AFM), the vertical force-distance curves (VFDC) are measured as follows (see Scheme 1). At first, the sharp tip of AFM cantilever is brought into contact with the sample layer to allow the molecule adsorption or chemical reaction on the cantilever tip. Then, the Z piezo moves the cantilever away from the surface. As a result, the molecule can be stretched between the substrate surface and cantilever tip. The stretched molecule exerts the force on the cantilever beam and bends it downward to the sample surface. During this process, the vertical cantilever deflection is detected optically. Usually, the AFM software allows user to control the point
of contact on the sample surface, the applied force at the contact, the Z piezo travel distance, the rate of piezo movement, the number of experimental points per force curve, the time delay between the measured points, and some other parameters depending on AFM. If bending force constant of AFM cantilever beam is known, then the collected cantilever deflection-distance curves can be converted into the force-distance ones. As a result, it is possible to obtain the quantitative information on the single molecule elasticity, the conformation changes, the chemical bond strength and other important properties.

However, we unaware of any literature data for the lateral force-distance curve (LFDC) studies (see Scheme 1). At the same time, there should be no principal experimental difficulties to perform such measurements with AFM. Moreover, the conventional AFMs usually have the XY piezo ranges several times larger than Z ones. Therefore, much longer macromolecules such as DNA and high molecular weight polymers can be stretched in lateral direction with AFM. Experimentally, it is also more convenient to study the different parts of elongated single macromolecule on solid surface by other microscopy/spectroscopy methods than vertically suspended one. The lateral pulling of molecule will always keep it in the focal plane of the microscope objective. Consequently, we report the experimental results on lateral stretching of single polymer molecule on solid surface up to several micrometers. We also discuss the nature of observed vertical and lateral deflection signals in our experimental setup and outline the future applications of lateral force-distance technique.

2. Experimental

SPMTools 5.01 package for Explorer AFM (ThermoMicroscopes) was used to develop our application in Visual Basic 6.0 environment for LFDC measurements (along Y axis, see Scheme 1). Such software allowed the simultaneous collection of left-right (L-R) and top-bottom (T-B) cantilever deflection signals in a course of lateral cantilever movements as well as ordinary VFDC collection. Also, it was possible to adjust the initial Z piezo position before the force curve measurements. Regarding sample preparation, from 10 to 20 mg of poly(ethylene glycol)-bis-thiol (SUNBRIGHT DE-200SH, NOF)
were dissolved in 5 ml of Milli-Q water. Then, the drop of such solution was applied on gold-coated surface of the chip part of Olympus OMCL-TR800PB cantilever chip used as a substrate. Depending on experiment, the chip surface was incubated for desired time or solution drop was dried naturally. Finally, the cantilever chip was washed with excess of Milli-Q water to remove any unbounded poly(ethylene glycol)-bis-thiol, HS-PEG-SH. The ANOPORE inorganic aluminum oxide membrane filter (20 nm pore size) was purchased from Whatman International Ltd. To collect the LFDC and VFDC, the longer lever of Olympus Bio-Lever cantilever chip was employed. All AFM experiments were conducted in Milli-Q water.

3. Results and Discussion

As it is seen from Figure 1, in the lateral deflection-distance curves (LDDC) of HS-PEG-SH in water, we clearly observed the rupture events from cantilever tip for the multiple (a) or single molecule (b, c) attachments. The original HS-PEG-SH length was about 145 nm. We believe that such long extension curves indicated that HS-PEG-SH self-polymerized under ambient conditions during sample preparation. Except the contact part in VFDC (see Figure 1(d)), the LDDC and VFDC showed very similar shape. In our repeated VFDC experiments, the rupture force, \( F_r \), never exceeded 1 nN. Since reported Au-S bond strength was about 1.5-2.7 nN [7,8], we observed only physical adsorption of polymer on cantilever tip. Probably, the terminated S-H groups were easily oxidized at ambient conditions during sample preparation that prevented the chemical bond formation between Au surface of the tip and S atom at the polymer end group.

Note that shapes of L-R and T-B photodetector signals in LDDC were virtually indistinguishable (Figure 1(a)). Since the cantilever tip in LDDC was at some distance from substrate surface, the stretched molecule exerted the lateral and vertical force components on the

![Scheme 2](image_url)
cantilever beam. Then, torsion and bending deformations of the beam can be detected depending on the corresponding cantilever force constants, the strength of applied force, and the photodetector sensitivities to the torsion, $S_{L-R}$, and vertical, $S_{T-B}$, cantilever deflections. Here it was reasonably assumed that XY in-plane cantilever deformations were small compared with torsion and vertical ones. To understand which of two signals, L-R or T-B, captured the actual cantilever deflection process during LDDC collection, the corresponding photodetector sensitivities were calibrated. The $S_{T-B} \approx 0.14 \text{nA/nm}$ was measured from the contact part of VFDC data collected on clean microscope cover glass surface in water (see linear fit in Figure 2(a)). The $S_{L-R}$ was estimated from the lateral friction-force loops measured for the cantilever in the direct contact with ANOPORE filter in water (see Figure 2(b,c)). In such measurements, the cantilever tip (30 nm radius) occasionally trapped in some filter pores during lateral movement. Note that vertical cantilever bending was restricted in such experiments. Indeed, for the tip height of 7 μm, $h$, and $y=40$ nm, the vertical cantilever displacement, $z$, due to the torsion should be only ~0.1 nm (see Scheme 2). By using estimated $S_{T-B}$ value, the absolute T-B signal change should be also very small (~0.014 nA). Therefore, the major cantilever deflection in the lateral friction-force loops at moderate deformations was torsion and the apparent large changes of T-B signal were due to the T-B vs. L-R cross-talk (see Figure 2(d)). As a result, $S_{L-R}$ can be calculated with a good accuracy from linear fits depicted in Figure 2(b,c). Next, for the typical rectangular cantilevers, the reported ratios, $k_t/k_n$, of torsional, $k_t$, to normal (bending), $k_n$, force constants were about $10^{2}$~$10^{3}$ [9,10]. Therefore, at $F_t = 0.6$ nN (see Figure 1(d)) and for $k_n = 0.006 \text{ N/m}$ (Bio-Lever), the expected cantilever displacements, $y = F_t / \left[ (k_t/k_n) y_n \right]$, should be 1~0.1 nm (see Scheme 2). Then, by using the estimated $S_{L-R} \approx 0.55 \text{nA/nm}$, the values of expected L-R signals due to cantilever torsion should be from 0.55 to 0.055 nA. Obviously, the LDDC depicted in Figure 1(a) differed not only by the sign of expected L-R signal change due to the cantilever torsion, but also by the magnitude of the absolute values at rupture events.

Therefore, in our LDDC measurements, the T-B photodetector signal was more appropriate to use since it captured the main cantilever deflection process. Apparent L-R signal registered with our AFM was merely a result of L-R vs. T-B cross-talk, which is a common problem of fixed optics scanning-by-probe AFMs [11]. The magnitude of the cross-talk was calibrated from Figure 2(d), where L-R is plotted versus T-B signal. Data were collected during the VFDC measurements on clean cover glass surface in water. The slope was ~0.43. Its value was very similar to that (0.46) shown in Figure 1(c) for LDDC measurements, which again clearly confirmed the L-R vs. T-B cross-talk. In summary, with our cantilever used in LDDC measurement of rupture forces up to 1 nN, the primary cantilever deflection was vertical bending. This observation greatly simplifies the quantitative analysis of LDDC since $k_n$ is usually known or it can be easily estimated by different methods in contrast to $k_t$.

Another interesting observation was the strength of the vertical force component at rupture events, $F_\perp$. For example, as it follows from Figure 1(c) and values of $k_n$ and $S_{T-B}$, $F_\perp$ was ~70 pN. In this case, the molecule was extended up to $y=2400$ nm at $d=50$ nm above surface. Then, from simple geometrical model shown in Scheme 3(a), the lateral force component,
\[ F_\perp = F_\parallel / \sin[\arctan(d/y)] \], along suspended molecule was \(~3\) nN. Note that this value was larger than Au-S bond strength. In some experiments, the \( F_\perp \) strength even up to \(~10\) nN can be deduced. Obviously, such large \( F_\perp \) values were unrealistic and reflected the inadequacy of the model shown in Scheme 3(a). Most likely, the molecule was stretched just on the surface with only small portion of its length been actually suspended as it is shown in Scheme 3(b). From recent theoretical work on response of an adsorbed polymer that is pulled by an AFM, the angle \( \alpha \) (see Scheme 3(b)) depends on the surface friction coefficient, the adsorption strength, the lateral cantilever velocity, the polymer length, and \( d \) [12]. As a result, the \( F_\perp \) and \( \alpha \) are self-adjusted by the surface-polymer interactions during the pulling. In principle, since adsorption strength could be estimated from VFDC, the friction coefficient could be also determined from LFDC experiments.

In conclusion, we demonstrated that lateral force-distance curve technique had a potential for keeping the extended single molecule on substrate surface as well as for the measurements of the friction coefficient between such molecule and surface.

References
[1] Burnham N A, Colton R J and Pollock H M 1993 Nanotechnology 4 64-80
[2] Cappella B and Dietler 1999 Surf. Sci. Rep. 34 1-104
[3] Butt H-J, Cappella B and Kappl M 2005 Surf. Sci. Rep. 59 1-152
[4] Hodges C S 2002 Adv. Colloid Interface Sci. 99 13-75
[5] Hugel T and Seitz M 2001 Macromol. Rapid Commun. 22 989-1016
[6] Zlatanovaa J, Lindsay S M and Leuba S H 2000 Prog. Biophys. Mol. Biol. 74 37-61
[7] Garnier L, Gauthier-Manuel B, van der Vegte E W, Snijders J and Hadziioannou G 2000 J. Chem. Phys. 113 2497-507
[8] Grandbois M, Beyer M, Rief M, Clausen-Schaumann H and Gaub H E 1999 Science 283 1727-30
[9] Green C P, Lioe H, Cleveland J P, Proksch R, Mulvaney P, Sader J E 2004 Rev. Sci. Instrum. 75 1988-96
[10] Cannara R J, Eglin M and Carpick R W 2006 Rev. Sci. Instrum. 77 053701-1-11
[11] Varenberg M, Etsion I and Halperin G 2003 Rev. Sci. Instrum. 74 3569-71
[12] Serr A and Netz R R 2006 Europhys. Lett. 73 292–8