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Single polymer chain rubber elasticity investigated by atomic force microscopy

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Abstract

A method, which is called as 'nanofishing', enabled us to stretch a single polymer chain adsorbed on an Au(111) substrate with picking it at its two thiol-modified termini using atomic force microscope equipped with a gold-coated probe. A force–extension curve obtained for a single polystyrene chain in a Θ solvent (cyclohexane) showed a good agreement with a so-called worm-like chain model, and thus gave microscopic information about entropic elasticity. Solvent effects on the statistical properties of single polymer chains were also investigated. For example, the second virial coefficient in cyclohexane was determined at a single polymer basis, which was almost comparable with a simple Flory's lattice model.

Keywords: Atomic force microscopy; Entropic elasticity; Virial coefficient

1. Introduction

In order to promote 'polymer nanotechnology', one must consider the following points: at first, observation tools with nanometer-scale resolution must be established. For surfaces and interfaces of nanometer-scale polymeric materials like polymeric nanoalloys and nanocomposite systems, scanning probe microscopy (SPM), especially atomic force microscopy (AFM) [1], has undoubtedly become indispensable. Secondly, nanometer-scale spectroscopy is the matter of vast importance. Again, AFM can be used to give material contrasts in terms of 'elastic' and 'energy dissipative' properties. Tapping-mode phase contrast and force modulation imaging techniques have been widely used for this purpose [2–7]. The final and most important point is that the physical (or any kinds of) properties must be quantitatively evaluated from the practical point of view. In this context, AFM is still a developing tool though many trials have been made to interpret obtained image contrasts to date.

In our group, several AFM analytical methods have been introduced to perform nanotribological [8] and nanorheological [9,10] investigations. By using these novel methods, 'energy dissipative' properties can be quantitatively estimated as well as 'elastic' ones. For example, time–temperature superposition principle was confirmed to be valid even at nanometer-scale by both tribological and rheological measurements. Elastic modulus and surface energy (or viscosity) were quantitatively and independently estimated by analyzing force–distance curves of melt-state surface.

With the above-mentioned methodologies, several applications become possible. Needless to say, because rubber elasticity is one of the most important physical properties of polymers, plenty of researches have been performed to understand it by experimental and theoretical approaches to date. Among them, for example, a long-believed assumption 'affine deformation' was proved not to be valid for a stretched natural rubber by ourselves [11]. Thus, AFM has become the most powerful tool to check many of traditional theories and believes. However, the most basic part, a single polymer chain elasticity, which is composed of entropic and enthalpic contributions, had mainly been the issue of theoretical frameworks and had not been investigated experimentally. It is because that an access to a nanometer-scale single polymer chain had almost been impossible before the invention of AFM.

According to literature, trials on the stretching of single polymer chains by means of AFM have been extensively performed by many researchers, which are called as

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‘nanofishing’ at present. However, these trials have been especially dedicated to studies of ‘single protein unfolding’ events [12–15], physisorbed macromolecules [16–20], polymer chains grafted on a surface [21], or a bridge composed of polymer chains [22]. In the former case, proteins with modified ends are sandwiched via specific chemical bonds between a substrate and a cantilever and can be successfully stretched in the course of force-curve measurement by AFM. Those successes are attributed mainly to two factors, proteins’ unique three-dimensional structures and the genetic engineering techniques for the modification of proteins’ ends. Comparing such cases, synthesized polymers have many disadvantages. For instance, it is difficult to attach any reactive groups at their ends in general. Thus, the force-curve data obtained to date have contained many complicated factors such that the adsorptions occur not only at tails but also at loops. The polydispersity also makes the situation much complicated. As will be described in this paper, however, a successful nanofishing result on the living-polymerized polystyrene (PS) chain with chemically active termini was obtained and thus several analyses could be conducted to investigate physical properties of a single polymer chain.

2. Experimental

A SH-terminated PS was used as a sample. It was based on a living-polymerized COOH-terminated PS with $M_n = 93{,}800$ and $M_w = 100{,}400$. The polydispersity was $M_w/M_n = 1.07$. The degree of polymerization was about 900 and thus its contour length was about 220 nm. The thiol groups were substituted for the COOH ends using 1,10-decane dithiol by means of thioester bonding with anticipating the preferential interaction between thiol and gold. The polyphosphate ester (PPE) was used as a condensation agent [23,24]. No dimer formation was indicated from the gel permeation chromatography (GPC) result. Au(111) surfaces on mica substrates were prepared as described [25]. The polymer was dissolved in a Θ solvent, cyclohexane, at 20 μM/ml, and 10 μl of the solution was cast on a Au(111) substrate. After 5 min of incubation, the surface was rinsed with a pure solvent.

All AFM experiments were performed by using NanoScopeIV (Veeco Metrology Group, USA). In order to pick up the SH-modified terminal, a gold-coated cantilever, OMCL-RC800PB-1 (OLYMPUS Co. Ltd, Japan), was used. Its resonant frequency was about 17 kHz (in ambient condition) and the nominal value of its spring constant was 0.11 N/m. At this moment, we did not measure the spring constant value experimentally. However, all the experimental results here were obtained by an identical cantilever to avoid statistical error caused by the usage of different cantilevers.

Force–distance curve measurements were performed in cyclohexane as schematically shown in Fig. 1. The force–extension curves were obtained directly from force–distance curves using the relationship that the z-piezo displacement can be divided into two quantities, cantilever deflection and polymer extension length.

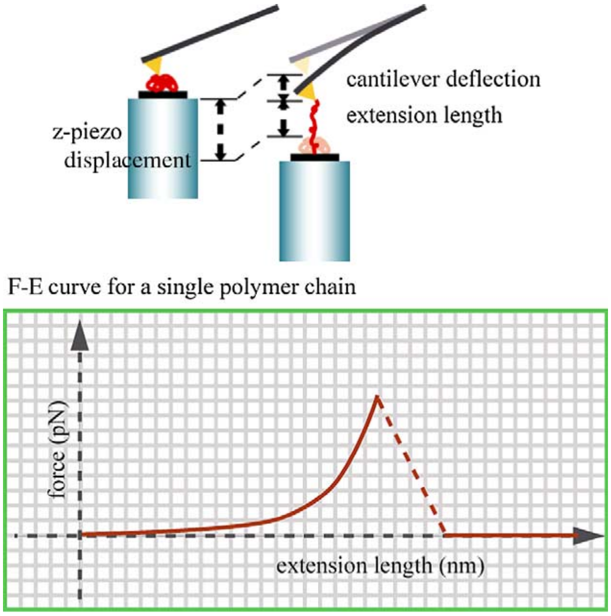

Fig. 1. The principle of nanofishing, a single polymer chain force–extension curve.

3. Results and discussion

Doted structures were observed on the gold substrate sufficiently apart from each other by the friction force microscopic (FFM) observation with Si_3N_4 probe as indicated by arrows in Fig. 2. We attributed these dots to individual polymer chains adsorbed and possibly taking random-coil forms [26], because the sizes of these dots were a few nanometers [27]. We also tried FFM observation with Au-coated probe (data not shown), resulting in the occurrence of streak lines with almost the same frequency, which might be related with dragging phenomena of single polymer chains. These results assured that the subsequent nanofishing measurements were basically on a single molecule basis.

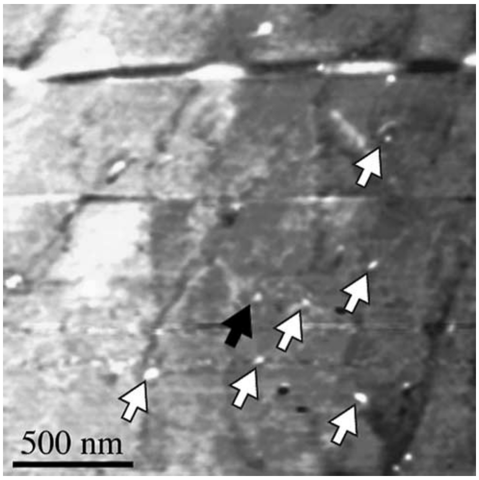

Fig. 2. A contact-mode AFM friction image (2 μm, observation in a good solvent, N,N-dimethylformamide) of individual PS chains possibly in random-coil form distributed on an Au(111) substrate.

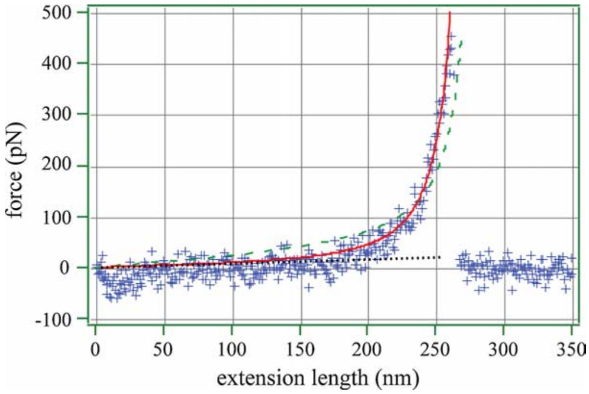

Fig. 3. Nanofishing of a single PS chain in cyclohexane. The solvent temperature was about 35 °C. The worm-like chain (WLC) and the freely-jointed chain (FJC) models were used to obtain fitting curves.

A typical force–extension curve measured in cyclohexane is shown in Fig. 3. The solvent temperature was kept at about 35 °C, which was corresponding to its Θ temperature for PS chains. Thus, a chain should behave as an ideal chain. At a glance, force data fluctuate much, however, this is due to thermal noise imposed on a cantilever. A simple estimation tells us that the rms noises in force signals both with ($\Delta F \sim 16.7$ pN for (δ; 0, 50)) and without ($\Delta F \sim 15.6$ pN for (δ; 300, 350)) a polymer chain attachment are almost comparable with thermal noise, $\Delta F = (k_0 k_B T)^{1/2} \sim 21.6$ pN, where k_0, k_B and T are cantilever spring constant, Boltzmann constant and temperature, respectively. This typical curve gave a nice fit (solid line) against the worm-like chain (WLC) model [28,29],

$$\frac{F l_p}{k_B T} = \frac{\delta}{L} + \frac{1}{4(1-\delta/L)^2} - \frac{1}{4} \tag{1}$$

where δ is extension length at external load of F. l_p and L are persistence length and contour length, respectively. The fitting results were $l_p = 0.31 \pm 0.01$ nm and $L = 284.5 \pm 0.8$ nm. The persistence length almost corresponded to a single monomer length. The discrepancy between rupture length of 260.9 nm and the contour length of 284.5 nm indicated that the polymer chain was not fully stretched at the rupture event. This was because the rupture event was stochastic process and depending on many factors such as pulling speed, bond strength, and temperature. Thus, we had to regard that l_p and L were intrinsic to polymer itself, while rupture length and force were not so. Then, the calculated degree of polymerization from these two intrinsic values became about 906, which was in good agreement with the expectation from the synthesis information. The slope at the lowest extension limit (dotted line in Fig. 3) was 1.20×10^{-4} N/m. The value should be a single polymer chain elasticity caused by entropic contribution. The simplest estimation was also performed with the following equation derived from Eq. (1) for the condition, $\delta \ll L$,

$$F = \frac{3 k_B T}{2 l_p L} \delta \tag{2}$$

The substitution of l_p and L resulted in the spring constant of 0.71×10^{-4} N/m for a single polymer chain elasticity, implying the measurement was consistently performed with theoretical predictions.

We also checked the validity of the freely-jointed (FJC) model as seen in other reports [30],

$$\frac{F l_K}{k_B T} = L^{-1}\left(\frac{\delta}{L}\right), \quad L(x) = \coth x - \frac{1}{x} \tag{3}$$

where l_K is Kuhn length and $L(x)$ is Langevin function. There is a famous relationship between Kuhn and persistence lengths, $l_K = 2 l_p$ at the limit of $\delta \ll L$. Fitting result was superimposed in Fig. 3 (dashed line). It was obvious that FJC model did not well represent the experimental result. The fitting results were $l_K = 0.21 \pm 0.04$ nm and $L = 281.5 \pm 20.4$ nm. The obtained Kuhn length was too small and, furthermore, the contour length had relatively large error. Thus, we tentatively concluded that FJC model was not applicable for the present case.

Although it was sufficiently narrow for the general purpose, the polydispersity of 1.07 does affect our observation, i.e. force–extension curves showed some fluctuations on the rupture length and the rupture force exerted and, therefore, the persistence length and the contour length. Fig. 4 shows the histogram of (a) persistence length and (b) contour length for 100-times nanofishing events. As seen in Fig. 4(a), fitted values for persistence length were sometimes too small, even smaller than C–C bond length. Thus, it was inevitable to further investigate this impossible result. We at first looked at

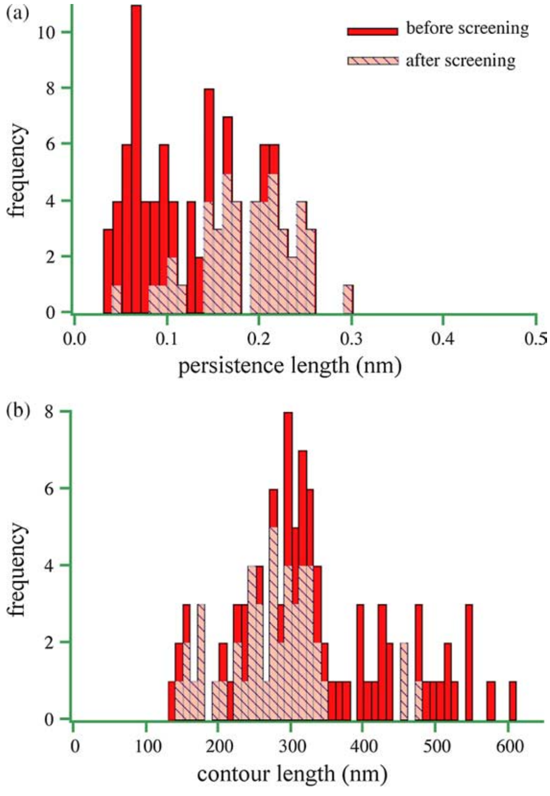

Fig. 4. The histogram of (a) the persistence length and (b) the contour length before and after screening (see the text more in detail). The solvent temperature was about 30 °C, slightly below Θ temperature.

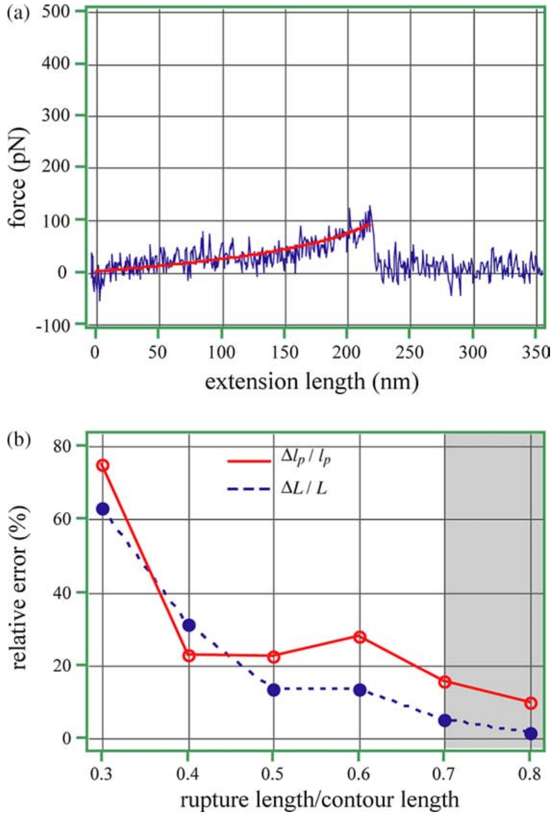

Fig. 5. (a) Failure nanofishing of a single PS chain. (b) The relationship on relative errors of persistence and contour lengths against the ratio between rupture length and contour length.

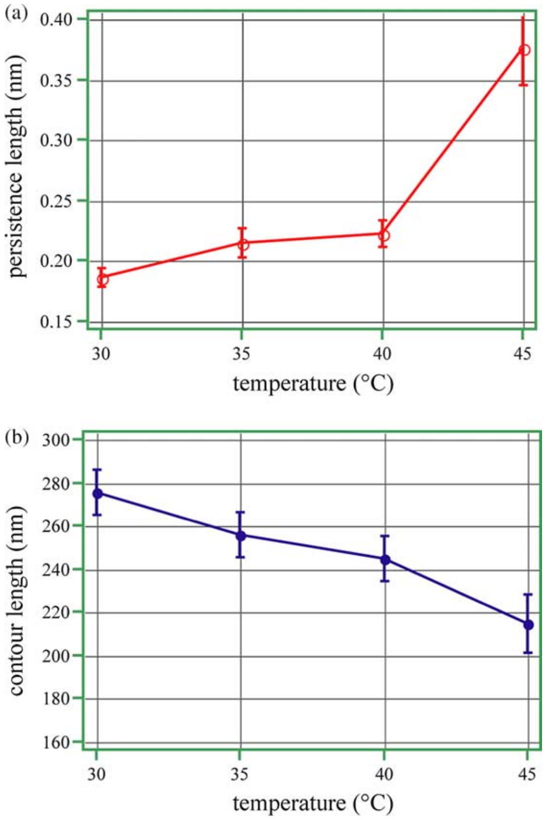

Fig. 6. The temperature dependences of (a) persistence length and (b) the contour length. The numbers of averaged constituents were 48, 62, 67 and 58 for 30, 35, 40 and 45 °C, respectively.

the original force curve as shown in Fig. 5(a). In this case, rupture event occurred with weaker rupture force, which resulted in the fitting parameters of $l_p = 0.09 \pm 0.01$ nm and $L = 367.0 \pm 23.0$ nm. In the case of failure experiments, persistence length tended to decrease and contour length increased. Thus, the ratio of the extension length at rupture against the fitted contour length became smaller (0.59 for Fig. 5(a)). As shown in Fig. 5(b), the relative errors for persistence and contour lengths proved to be a function of this ratio. The smaller the ratio became, the larger these errors became. In other words, successful nanofishing should provide a small deviation between rupture and contour lengths (0.92 for Fig. 3). Again, synthesized polymers usually own polydispersity. In addition, rupture event is essentially stochastic process. Thus, it is almost impossible to avoid a possible failure in fishing of polymer chains, which might discourage people to use nanofishing technique. However, we can also perform a certain screening by judging the ratio between rupture and contour lengths. From now on, we will proceed to the statistical analysis on a number of nanofishing results. It was regarded that a nanofishing event with the ratio being less than 0.7 was a failure one and thus omitted from the population for further analysis. Going back to Fig. 4, the histograms after this screening process were added. The number of population (originally 100 events) decreased to be 48.

Fig. 6(a) shows the temperature dependence of the persistence length. At each temperature, several tens of fishing results were averaged. The error bar was defined as $\sigma/\sqrt{N}$, where σ is standard deviation and N is the number of averaged constituent in each temperature. Note that the error level of each persistence length is negligible compared with this error and that the artifacts caused by failure nanofishing had already been omitted. Thus, the error bar was purely due to polydispersity. It was possible to observe some change in the persistence length in the figure. If the fishing was operated in cyclohexane at temperatures deviated from its Θ temperature of 35 °C, some effects caused by monomer–solvent interaction can be emerged. It is so-called excluded volume effect [31]. Thus, we can conclude that nanofishing can reveal the monomer–solvent interaction in a single polymer chain.

Since, our experimental results were fitted with a simple WLC model, a solvent effect would be incorporated in the value of the persistence length. According to Flory's theory, the mean square of the end-to-end distance, R, becomes

$$\langle R^2 \rangle = nb_\theta^2 \left[1 + \frac{4}{3}z + \cdots \right] = nb^2 \tag{4}$$

where n is the degree of polymerization and b_θ is the bond length for an ideal chain (Θ-state). b is an apparent bond length in the case of nonΘ-state. The value z represents the second virial coefficient, i.e. two-body interaction and expressed as follows,

$$z = \beta n^{1/2}\left(\frac{3}{2\pi b^2}\right)^{3/2} = \frac{4\pi a^3}{3}\left(1-\frac{\theta}{T}\right)n^{1/2}\left(\frac{3}{2\pi b^2}\right)^{3/2}$$

$$= \sqrt{\frac{6}{\pi}}\left(\frac{a}{b}\right)^3\left(1-\frac{\theta}{T}\right)n^{1/2} \qquad (5)$$

where a stands for the size of solvent. For simplicity, if we put the assumption $a=b$ (the lattice model),

$$z = \sqrt{\frac{6}{\pi}}\left(1-\frac{\theta}{T}\right)n^{1/2} \qquad (6)$$

Now, we consider that b can be regarded as the persistence length, l_p, then, we obtain

$$\left(\frac{l_p(T)}{l_p(\theta)}\right)^2 = 1 + \frac{4}{3}\sqrt{\frac{6}{\pi}}\left(1-\frac{\theta}{T}\right)n^{1/2} \qquad (7)$$

.

The substitution of $\theta=308$ K and $n=900$ results in the following relationship,

$$\left(\frac{l_p(T)}{l_p(\theta)}\right)^2 = 56.3 - 17{,}032\frac{1}{T} \qquad (8)$$

.

Fig. 7 is the result of the least square curve fitting against the data in Fig. 6(a). The obtained values were

$$\left(\frac{l_p(T)}{l_p(\theta)}\right)^2 = (44.7 \pm 19.9) - (13{,}410 \pm 6170)\frac{1}{T} \qquad (9)$$

.

Our first result of virial coefficient determination contained large errors, which must be overcome in the future. However, one can think about the difference was not so serious because the experiment conducted here was on a single polymer chain basis. Thus, we can conclude that our nanofishing is well described by classical Flory's model.

There remain several unsolved questions. The first one is that the assumption of the lattice model is merely the simplest approximation, where the individuality of polymer chain is lost. Our future effort must be dedicated to the comparison of our results with a light scattering experiment for PS chains with almost the same molecular weight [32]. Such a comparison will provide a quantitative measure of excluded volume size. We also have to conduct the same measurements on different molecular weight samples in order to discuss the validity of many theoretical predictions including scaling concept introduced by de Gennes [33].

The second more practical problem is whether WLC model is adequate to explain the observed phenomena or not. As shown in Fig. 6(b), the contour length did show a certain temperature dependence, which was unexpected result. Even assumed that rupture event has a strong dependence on temperature, contour length should be correctly estimated if the curve fitting was perfectly successful. Therefore, our tentative speculation is that the separation between persistence length and contour length was not perfect in the case of WLC model. Further studies are necessary to elucidate the point. As a similar consequence, the estimation of persistence length should have some mismatch with a realistic value. Actually, one may felt that our persistence lengths were too short, to which at this moment we also cannot give any clear answer. As discussed at Fig. 5(b), the relative error had some correlation with the fitted value of contour length. In point of fact, persistence length itself had such correlation as shown in Fig. 8, where persistence length became larger with increasing ratio between rupture length and contour length (data contains failure nanofishing results). Some theoretical support will be essential to solve the problem, while the situation is not so simple as discussed in the next paragraph. In any case, we have concluded that the usage of WLC model must be regarded as the first approximation.

As shown in Fig. 9, the strange conformational jump was sometimes observed. In this paper, we only discussed about entropic contribution of a single polymer chain elasticity. However, this type of enthalpic contribution should be very important to understand the real world. Any theoretical development in the future should consider the point. WLC or FJC models does not concern it and, therefore, we should recognize that the applicability of these models is very much limited.

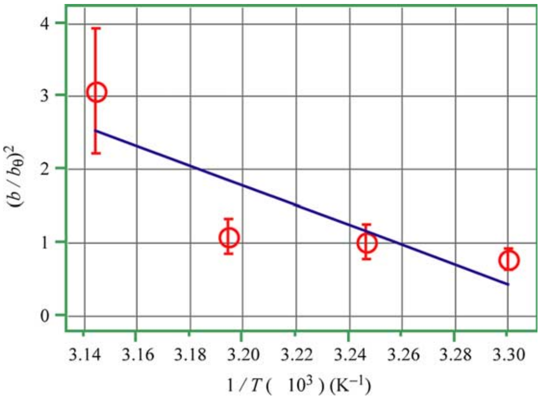

Fig. 7. The determination of the second virial coefficient of a single PS chain in cyclohexane. The solid line represents the least-square curve fitting result.

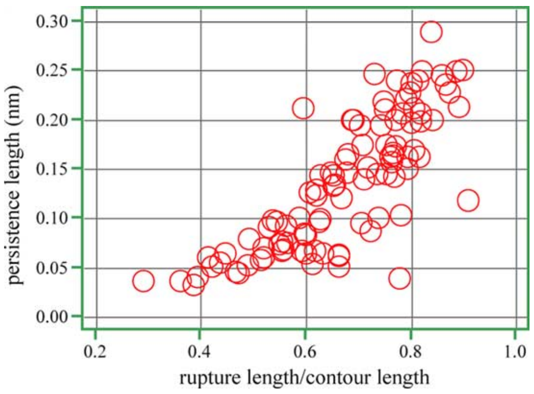

Fig. 8. The correlation between persistence length and the ratio between rupture length and contour length.

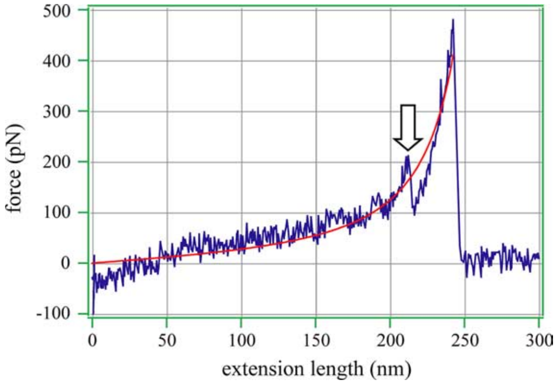

Fig. 9. A strange conformational jump sometime observed during nanofishing.

4. Conclusion

'Nanofishing' realized by AFM gave a force–extension curve for a single polystyrene chain with thiol termini in a Θ solvent (cyclohexane), which showed a good agreement with a so-called worm-like chain model to some extent, and thus gave microscopic information about entropic elasticity. The second virial coefficient in cyclohexane was determined at a single polymer basis. The method will unveil hidden properties of polymer chains or polymer solutions by any macroscopic measurements in the future.

Acknowledgements

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