

AFM Evidence of Rayleigh Instability in Single Polymer Chains

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We present experimental evidence of the Rayleigh–Plateau instability of a single chain in poor solvent conditions using single molecule force microscopy. Poly(*N*-isopropylacrylamide) (PNIPAM) and poly(ethylene oxide) (PEO) are adsorbed onto silicon nitride surfaces in various solutions corresponding to poor and good solvent conditions. In good solvent conditions, the force–separation profile is identical to that described previously and attributed to the elastic stretching of single polymer chains. However, in poor solvent conditions, we see a dramatically different force profile, characterized by steps or plateaus of constant force. These plateaus represent the “pull-out” of chain segments from collapsed globules of polymer collected at each of the separating surfaces. A statistical analysis of the large number of force profiles collected indicates that these plateaus are quantized, suggesting pull-out of several chains of different length. Moreover, the frequency of the steps suggests that we can distinguish pulled loops from pulled tails.

I. Introduction

The manipulation of an individual polymer chain adsorbed to a surface has received considerable attention in the past 5 years from both theoreticians and experimentalists. With the advent of atomic force microscope (AFM) and optical/magnetic tweezers scientists are able to impose nanometer scale deformations and measure forces on the scale of piconewtons. AFM has been used to investigate not only the adhesion and elasticity of individual polymer chains^{1–10} but also domain unfolding in biopolymers.^{11–13} However, this previous work focuses almost exclusively upon the good solvent case where the chains are swollen by solvent and form loose coils. Under these conditions, polymer tails or loops can be “grabbed” by an adsorbing AFM tip. Separation of the AFM tip and the surface produces a tension in the bridging polymer which is measured and characterizes the elasticity of the chain. The stretching at weak extensions is purely entropic, but at higher extensions, there is an additional

enthalpic cost of straightening the chain. Such stretching forces obtained from AFM data have been fitted to various models, including freely jointed chain (FJC)¹⁴ and worm-like chain (WLC)¹⁵ models, along with variations of these.¹⁶ These stretching forces increase monotonically with extension until the adsorption site or monomer–surface contact is broken at extensions on the order of hundreds of nanometers. Recently, force profiles of an oligomer of ethylene oxide were constructed using ab initio calculations.¹⁷ However, the chain extension was limited to a few angstroms, which is much smaller than the hundreds of nanometer extensions in our AFM experiments.

Much less attention has been placed upon the stretching of single chains in poor solvent. The stretching of chains which are collapsed in poor solvent was first studied theoretically by Halperin and Zhulina,¹⁸ who argued that at weak extensions the globule deforms into an ellipse and then into a cylinder. At a critical extension the polymer undergoes a sharp first-order transition into a “ball–string” configuration. This transition is driven by the high surface tension under poor solvent conditions. In this way, the transition is analogous to the surface tension driven breakup of a column of liquid into a series of droplets, referred to as the Rayleigh–Plateau instability.¹⁹ However in the polymer case, the applied tension draws out a thin filament rather than a series of disconnected droplets, owing to the constraint of connectivity of the monomers. The force required to pull the chain beyond this critical extension, or to “pull-out” the chain monomer-by-monomer from the collapsed globule, is constant and independent of extension. Computer simulations^{20,21} of the extension

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of a single polymer chain in poor solvent have verified the existence of this ball-string configuration.

In this paper we present for the first time experimental evidence of the Rayleigh instability of a single chain using single molecule force microscopy. In a simple experiment, we adsorb polymer onto a flat surface and use the adsorbing tip of an AFM to probe the polymer. This involves repeatedly bringing the surface and AFM tip into contact and separating them again, while simultaneously measuring the force on the tip. This results in chains forming bridges between the two adsorbing surfaces. The resulting force versus separation profile is often relatively featureless, showing only a primary adhesion upon retraction. However, over many separation cycles we find two distinct types of force profiles, depending upon the solvent conditions. The characteristics of the first type of force profile are identical to that observed by previous authors and have been interpreted as the elasticity of various polymers.^{1-8,10-13} This characteristic force profile is referred to as a "Langevin" event as it has been fitted to various elasticity models. In poor solvent, we see a new, second type of profile. It is characterized by "steps" or plateaus of constant force which extend over separation distances which are comparable to the Langevin events. At these separation distances, polymer-solvent contact is minimized by the formation of surface-bound polymer globules at each surface, connected by a polymer filament, Figure 1. This is the analogue of the ball-string configuration of the single chain Rayleigh instability. As the surfaces are separated further, monomers are pulled out of the surface bound globules and incorporated into the lengthening filament. The force of extension of the filament, measured as the force on the AFM tip, is constant over the separation distance, reflecting the constant rate of monomer extracted from the surface bound globules. This polymer filament may contain several chains, and surface separation can completely pull-out the shorter chains. With each pull-out, the plateau force drops discontinuously in a "steplike" manner, until the last single chain in the filament is pulled out and the force between the surfaces returns to zero. We refer to such constant force events as "Plateau" events after Plateau's work in instabilities as well as the physical description of the force versus distance profile.

The remainder of the paper is organized in the following manner. In the next section we describe the chains investigated, poly(*N*-isopropylacrylamide) (PNIPAM) and poly(ethylene oxide) (PEO), and the AFM experimental procedure. In section III we present force profiles for single chain PNIPAM stretching, focusing first upon the Langevin events observed in good solvent conditions and then the new Plateau events observed in poor solvent. For the PNIPAM experiments, the solvency condition is changed in situ by elevating the temperature of the aqueous solvent above the lower critical solution temperature. PEO in various concentrated electrolyte solutions provides another view of the same phenomena, and these PEO results are presented and discussed in section IV. Although the PEO profiles are very similar in character to those of the PNIPAM profiles, the PEO profiles contain a much larger number of Plateau events which allow a statistical analysis. From this we find that the plateau force is quantized and that the frequency of these quantized steps shows an interesting pattern. This implies that we can distinguish the number of chains that are being pulled in poor solvent and distinguish loops from tails by the frequency of Plateau events.

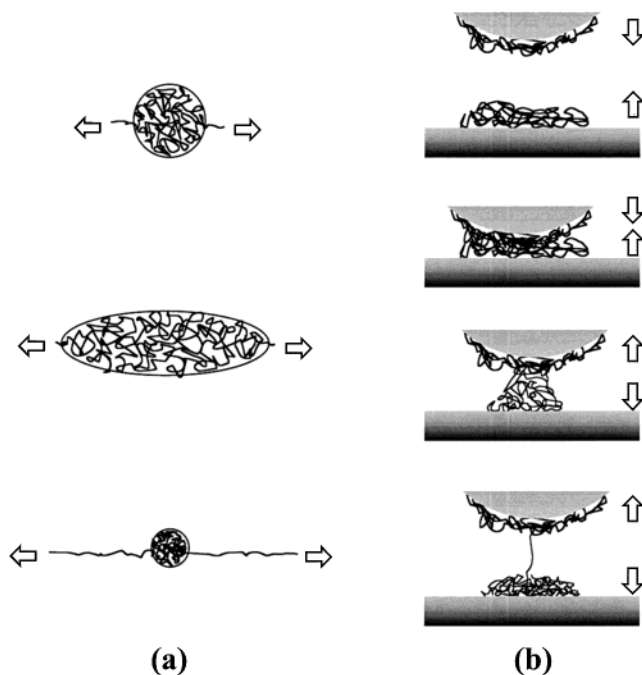


Figure 1. Schematic of the Rayleigh instability for a single polymer chain in a poor solvent. Theory and simulation show that the extension of the ends of a collapsed chain or globule leads to the configurations depicted in (a). The collapsed polymer or globule deforms into an elliptical shape and, at a critical extension, forms a "ball-string" configuration. Extension of the strings from the ball occurs with a constant force. These configurations arise due to the high polymer-solvent interfacial energy. Experimentally, it is difficult to "grab" the ends of a single chain; so we use (b) a flat surface and an AFM tip to produce an analogue of the Rayleigh instability. The surface-adsorbing polymer is sandwiched between the flat surface and AFM tip. Upon separation the polymer "necks", forming a bridge between the surfaces, and at a critical separation, the high surface energy favors the formation of surface-bound globules which are connected by a polymer filament. This filament can contain one or a few chains. Further separation draws monomers out of the globules and into the filament. The surface energy penalty is proportional to the length of the filament, and consequently, the extensional force is constant and independent of surface separation.

II. Experimental Section

A. Materials. Two different model polymers were studied: poly(*N*-isopropylacrylamide) (PNIPAM); poly(ethylene oxide) (PEO). PNIPAM was synthesized according to Zhou et al.,²² precipitated twice from acetone/*n*-hexane, and confirmed using ¹³C NMR. The molecular weight, determined by GPC, is $M_w = 9.0 \times 10^6$ and is quite monodisperse ($PI \approx 1.00$). PEO with an average molecular weight of 10^6 was purchased from Aldrich Chemicals and purified by reprecipitation twice from dichloromethane/diethyl ether. The salts used in this study, K_2SO_4 and KNO_3 , were purchased from Merck Pty. Ltd. and BDH Laboratory Supplies, respectively, and used without further purification.

The aqueous solutions of PNIPAM and PEO were prepared by gentle shaking in Milli-Q water for approximately 12 h, diluted to about 0.001 and 0.3 wt %, respectively, and filtered through prewet 0.2 μ m Teflon membranes. For our studies of PEO in salt solutions, K_2SO_4 or KNO_3 was added to make the salt concentration 0.45 and 0.25 M, respectively and filtered prior to injection. The polymer solution was injected into the AFM fluid cell from which measurements are made at a specified and controlled temperature. The base of the fluid cell is chemical vapor deposited silicon nitride²³ that was water-plasma treated immediately prior

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to the experiment to ensure clean surfaces amenable to polymer adsorption. Both of the polymers readily adsorb via H-bonding to the silicon nitride surfaces. Additionally, both are above their glass temperature temperatures and hence have sufficient mobility to vary their degree of surface aggregation.

The polymer solutions were allowed to equilibrate in the fluid cell for 1–2 h before flushing with Milli-Q water at the same temperature. In the case of the salt studies, we flushed the cell with filtered 0.5 M K_2SO_4 or 0.25 M KNO_3 solution. This final flushing removes excess and poorly adsorbed polymer and results in a sparsely covered surface.

B. Equipment and Technique. Force measurements were made using a Digital Instruments NanoScope IIIa force microscope which was thermally isolated by placing a large inverted vacuum dewar over the instrument. By the circulation of water through a copper coil around the microscope head, the desired temperature can be maintained within 0.1 °C. In this way, we are able to maintain the temperature of the polymer system as it adsorbs to the substrate, throughout equilibration, and during the force measurements. Details of the force measurements are found elsewhere. Briefly, the force measurements are obtained by bringing the tip of AFM cantilever into contact with the polymer covered substrate. As both substrate and cantilever tip are made of the same material, polymer is adsorbed onto both surfaces. Upon separation or retraction of the surfaces, the polymer can bridge the two surfaces and the tension in this bridge causes a deflection in the cantilever spring which is monitored by the deflection of a laser beam reflected from the back of the cantilever. For each cantilever used, we determined the spring constant in situ using a hydrodynamic method²⁴ which allowed us to convert the deflection versus surface separation signals to force versus separation profiles. The spring constants varied from 0.019 to 0.062 $N\ m^{-1}$ (or 2–6 $pN\ \text{\AA}^{-1}$), and the cyclic rate of approach and retraction of the surfaces was set at 1 Hz, corresponding to speeds of several hundred nanometers/second. Higher frequencies (10 Hz) did not affect the character of the force profiles.

III. Force Profiles of PNIPAM

PNIPAM has an aqueous lower critical solution temperature (LCST) of 31–34 °C;²⁵ that is, water is a good solvent at temperatures below the LCST and a poor solvent at temperatures above it. Thus, we are able to vary the solvent quality in situ by changing the temperature within this convenient range. Changes in the force profiles can then be attributed solely to the solvation conditions. Typical force profiles resulting from single chain stretching as the cantilever and the substrate are separated are shown in Figure 2. In this and the following profiles, we use the convention that negative forces represent tension in the bridging polymer or an attractive force between the tip and substrate while a positive force represents repulsion between the surfaces. Figure 2a shows a force profile obtained for aqueous PNIPAM at 12 °C below the LCST, well within the good solvent regime. Note that within the first ~50 nm of separation the force between the tip and substrate is strongly attractive or adhesive. This primary adhesion is nonspecific and masks any forces which can be attributed the tension of a chain within this region. Following this primary adhesion, and in about 10–15% of the force profiles, we observe the typical saw-tooth features which have been attributed to single chain stretching. Each saw-tooth represents the stretching of a chain of fixed number of monomers which bridge the two surfaces: at weak extension the tension in the chain is linear with separation, but as separation approaches the contour length of the chain, the force increases sharply until, at a sufficiently large force, one end of the chain detaches from the surface and the tension abruptly

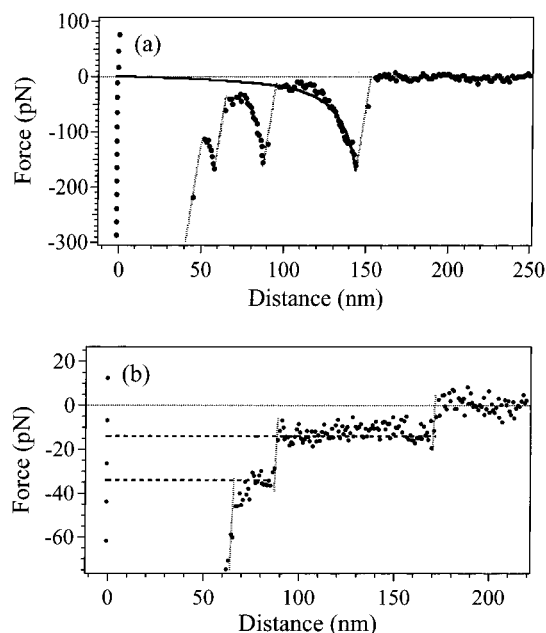


Figure 2. Force versus distance for PNIPAM in aqueous solution at temperatures corresponding to (a) good and (b) poor solvent conditions. For profile a, the temperature is maintained at 22 or 12 °C below the LCST and corresponds to good solvent conditions in the bulk. The profile shows multiple Langevin events which arise from the simultaneous stretching of three bridges of different size. Each of these bridges is comprised of a sequence of monomers whose ends are fixed at each of the surfaces. We show a fit of the WLC model¹⁵ to the longest bridging chain (black solid line). The fitting parameters are L , the contour length of the bridge, and a , the persistence length. The fitted contour lengths and persistence lengths (L , a) in nanometers for the three Langevin events are (80.8, 0.1), (99.4, 0.5), and (164.6, 0.4). These bridges detach at an average of around 72–88% of their contour length. In profile b, the temperature is maintained at 39 °C, which is above the LCST and corresponds to poor solvent conditions in the bulk. This profile shows multiple Plateau events, each event corresponding to pulling a chain monomer-by-monomer from a surface-adsorbed globule. The complete pull-out of the free end of the chain from the globule corresponds to the discontinuous decrease or step in the plateau. The dashed lines are a guide to the eye to highlight the Plateau events. In both (a) and (b), the discontinuities in the force profile have been connected by a gray line of a fixed slope taken to be the spring constant of 0.019 $N\ m^{-1}$. The rms of the baseline noise is 11.0 pN

disappears. This detachment force is, for the PNIPAM system, around 100 pN. Such force profiles have been fitted to a number of force laws, among them the Langevin force function and variations thereof. For that reason we refer to such saw-tooth features as “Langevin” events as it signals the stretching of single chains of fixed contour length. Figure 2a is indicative of the simultaneous extension of three loops or bridges of different size. As the surfaces are separated, the extensional force of the smallest bridge is dominant while the others remain slack. The stretching force of the smallest bridge grows monotonically until it becomes highly extended and breaks around 60 nm. From this point the tension in the second bridge grows, releasing at 90 nm. The third bridge detaches at 140 nm. These three bridges may or may not be part of the same chain. That these Langevin events can be fitted to a single force law has served as evidence that these forces are attributable to single chains. In general, such force laws are of the form $f \sim g(x/L, a)$, where f and x are the measured force and imposed surface separation and L and a are fitting parameters associated with the contour length and persistence length of the chain.

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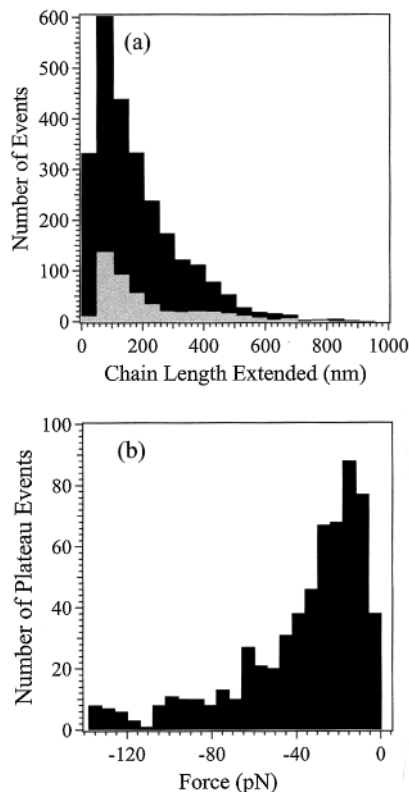


Figure 3. Histograms summarizing the statistics of force events for PNIPAM. Histogram a shows the frequency of maximum extension distances for both Langevin (black) and Plateau (gray) events that occur under both good and poor solvent conditions for a data set consisting of 1300 force profiles with a bin size of 50 nm. Histogram b shows the frequency of the mean force for plateau events collected from 500 force curves, each collected in poor solvent. The bin size is 6.0 pN.

Whether any Langevin event is due to a loop or a tail *cannot* be determined: the tension in a “grabbed” loop is attributed to the shorter side of the loop and cannot be distinguished from the tension of a pulled tail.

When the experiment is conducted under poor solvent conditions, a new type of event is observed in the force profile, a *distance-independent* force or plateau which is typically 1 order of magnitude smaller than the detachment forces observed in good solvent conditions. Figure 2b shows a representative force profile for the aqueous PNIPAM at temperatures equal to or greater than the LCST for the polymer. These constant force plateaus persist over an extension range (50–600 nm) that is similar to Langevin events, suggesting that these Plateau events are also due to single polymer chains. Such Plateau events occur more frequently with increasing temperature (toward poor solvent conditions) but disappear when the temperature is decreased below the LCST or under good solvent conditions. Figure 3 gives some statistics of the force profiles of PNIPAM in poor solvent conditions.

When imaging the surface in contact mode below the LCST (in good solvent conditions), the tip simply applies too great a shear force and displaces the adsorbate polymer. On occasion we are able to image single chains on the surface, but this is rare as the attractive tip can simply displace the polymer. However, as the temperature is increased above the LCST, aggregates or surface-bound “globules” of a few hundred nanometers in width and 10–20 nm in height are observed, Figure 4. These are the result of collapse of several chains into solvent-free surface-bound globules. The effective radius of an AFM tip is

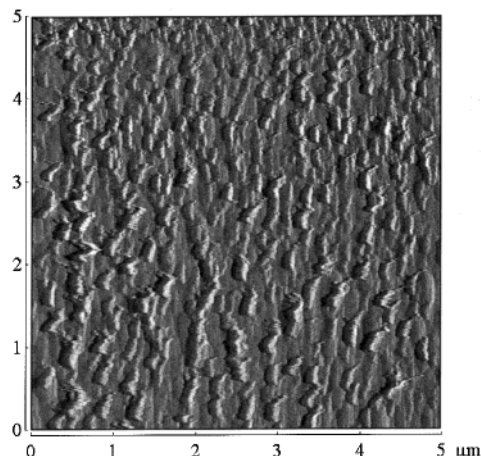


Figure 4. A $5\mu\text{m} \times 5\mu\text{m}$ AFM image of PNIPAM adsorbed to a silicon nitride surface at 42°C . The AFM tip was scanned horizontally, or perpendicular to the globules of collected polymer. The maximum height of the globules is ~ 35 nm, with most globules being 10–20 nm in height.

typically 1–30 nm,^{26–28} which is much smaller than the cross section of the surface adsorbed globules. In both imaging and force measurements, the repeated mechanical contact of the tip with the surface aids in collecting and concentrating the polymer into these globular aggregates. Clearly after the tip has scanned a region, that area no longer represents the original state of adsorption. However, this facilitates the formation of the polymer aggregate in the zone of contact.

We can understand these Plateau events, in the most general sense, in terms of a chain pulled monomer-by-monomer from an attractive potential to a zone of zero mean potential with minimal stretching. The filament of polymer between the surfaces is made up of monomers which have been pulled out of a local attractive potential. This bridge grows in number of monomers or chain length with surface separation. Stretching is minimal, as suggested by the small magnitude of the force plateau in comparison to the larger stretching forces in Langevin events. In principle, the plateaus can be attributed to one of two energy changes upon extension: (a) the destruction of attractive monomer-surface interactions as the monomers of the chain are “ripped” off the surface; (b) the creation of unfavorable monomer-solvent contacts, characterized by the interfacial tension, as monomers are pulled from the globule into the poor solvent. Although it is difficult to distinguish absolutely between these energies in a force plateau, evidence supports that the PNIPAM plateaus characterize the polymer/solvent surface tension. First, these Plateau events occur only under poor solvency conditions. Second, the force attributable to surface adsorption, that is the force of detachment, has been shown in good solvent to be around a few hundred piconewtons, much larger than the few tens of piconewton plateaus observed in the poor solvent/PNIPAM profiles. Last, but importantly, Langevin events, which signal the stretching of a chain which is end-adsorbed to *both* tip and substrate, are also seen alongside Plateau events in force profiles at poor solvency. This suggests that a Plateau event is associated with pull-out of a chain or loop having one end free or unadsorbed to the surface but embedded in the

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globule. In contrast, a Langevin event is associated with stretching of chains or loops having both ends fixed or adsorbed. Under good solvent conditions, there would be no force for extension of chains with only one end attached to the AFM tip; but in poor solvent an interface is formed between collapsed monomer and poor solvent and work must be done to transfer monomer from globule to filament.

In any single molecule force measurement, there is always a degree of ambiguity as to the number of chains or loops which are extended. In the case of Langevin events, the fitting of scaled force profiles is taken as sufficient evidence for single chain or loop extension. For the Plateau events here, it is particularly difficult to establish if the plateaus are associated with single chains or bundles of chains, precisely because the force of pull-out is of comparatively small magnitude and is featureless, i.e., it is independent of extension. However, the PNIPAM profiles do provide some significant clues. First, if we take the pull-out strand to be a cylinder of radius r and the surface energy to be γ , then the energy of pull-out to extension L is $2\pi rL\gamma$. Or equivalently, the force is $2\pi r\gamma$. For PNIPAM, we can adopt a value of γ between 40 and 45 mJ/m²,²⁹ which is the range of literature values for PNIPAM. This, together with our measured plateau force of the order of tens of pNs gives a cylinder of radius r on the order of angstroms; i.e., the cylinder is of molecular dimensions. This suggests that the Plateau event characterizes the pull-out of single chains from the surface-bound globule. In addition, we should expect to see stepped force plateaus corresponding to the simultaneous pull-out of multiple filaments. For example, if we are pulling simultaneously 3 filaments from the globule, then the force plateaus should drop discontinuously from $-F$ to $-2/3F$ and then to $-1/3F$ and finally to 0 with the complete pull-out of the different sized filaments. Figure 2b shows 2 plateaus, although the first plateau terminates at 90 nm, just outside of the primary adhesion zone. This plateau is indicative of a bridge that is completely pulled out of the globule by 90 nm, while a second bridge persists until the surfaces are separated to 170 nm. However, with over 1300 force profiles examined, the PNIPAM system did not provide sufficient statistics to verify quantized plateaus. Plateau events were rare in the PNIPAM system; for every Plateau event in the set of 1300 profiles there were 10 Langevin events. This might be due to the high affinity of the polymer to the silicon nitride surfaces increasing the chance that chains span both surfaces. In the following section, we describe similar experiments with PEO. This system is advantageous in that there were more Langevin and plateau events recorded in the force profiles.

VI. Force Profiles of PEO

PEO has an aqueous LCST of 96 °C,³⁰ which is not as easily accessible in AFM experimentation. However this critical temperature can be lowered by the addition of various salts, notably K₂SO₄ and KNO₃, to as low as 34 °C. Thus, we can change solvency by the addition of salt as well as by temperature. We consider, first, salt and temperature conditions for which Plateau events occur alongside Langevin events; i.e., conditions approaching or at poor solvency. Figure 5a shows a force profile of PEO in 0.45 M K₂SO₄ at 25 ± 2 °C, selected from a data set of 600 profiles. The force profile shows stepped plateaus, at least 3, arguably 4, extending beyond the primary adhesion

region. Many other force profiles in the data set show stepped plateaus, exclusively; however, many more contain both Langevin and Plateau events. The coexistence of both events typifies these systems and indicates that the stretching of doubly end-tethered chains occurs alongside the pull-out of singly end-tethered chains. Figure 5b is more typical as it shows a Langevin event followed by a Plateau event. The Langevin event, or stretching of a fixed loop or tail, persists until an extension of 120 nm at which point the chain detaches from one of the surfaces. Simultaneously, there is pull-out of a filament, and the force of pull-out, roughly 45 pN, offsets the stretching force. As this figure shows, it is relatively simple to identify and separate Langevin events from Plateau events and to construct a statistical analysis of the Plateau events. From the set of 600 force profiles collected from the PEO/K₂SO₄ system, we have identified over 400 Plateau events in the following way. The distribution of measured force within a candidate Plateau event is fitted to a Gaussian to find the mean force and standard deviation. The acceptance criteria for a Plateau event is that the standard deviation from the mean is less than 1.7 pN. We find that Plateau events occur with the same frequency as Langevin events; however, multiple plateau events are more likely than multiple Langevin events. Both types of events occur over extensions ranging from 50 nm (the edge of the primary adhesion zone) to 1100 nm.

Figure 5c provides the statistics on the mean force evaluated over the length of the Plateau events from the complete set of 600 force profiles. Plateaus of 55 pN are the most frequent, with significant populations at double that force or 110 pN, at 185 pN and a discernible population at 250 pN. Thus, the statistics of a large number of analyzed force profiles provide evidence of the description suggested by the profile of Figure 5a: Plateau events are quantized. In addition, there is a discernible population (17 in number) of plateaus of magnitude ~25 pN. This fits with our interpretation of multiple chain pull-out: if we attribute the pull-out force with the highest frequency to the extension of a single loop, then the 25 pN plateaus represent the less likely extension of a single tail. The 25 pN plateau population should also include the short-range extension of an uneven loop where monomers are extracted from the globule on one side of the loop, the other side being slack. The population of Plateau events of roughly 110 pN would then correspond to the extension of two loops. The populations of force plateaus centered at larger forces are more broad and reflect the pulling of more chains. The inset to Figure 5c is the autocorrelation of the frequency of plateau forces. Despite having only 400 Plateau events, the peak in the autocorrelation near 50 pN lends further evidence of quantization.

Other PEO studies with different solvent conditions provide fewer profiles and/or fewer plateau events but show similar results. Figure 6 is a typical profile for aqueous, no-salt PEO at 30 °C, and Figure 7 is a profile taken for aqueous PEO with 0.25 M KNO₃ at room temperature. These profiles show single and double plateaus, respectively, and each figure provides a histogram of the mean force of plateau events. The maximum extension over which these systems gave Langevin or Plateau events, (the extension at which the force returned to zero) was less than half that of the PEO/K₂SO₄ system. Consequently, for the same number of force profiles, there are far fewer events and the statistical analyses are correspondingly not as clear. The mean force of Plateau events of the aqueous no-salt PEO is predominantly on the order of ~55 pN with a significant population of plateaus with larger force, at roughly 100 pN. Although

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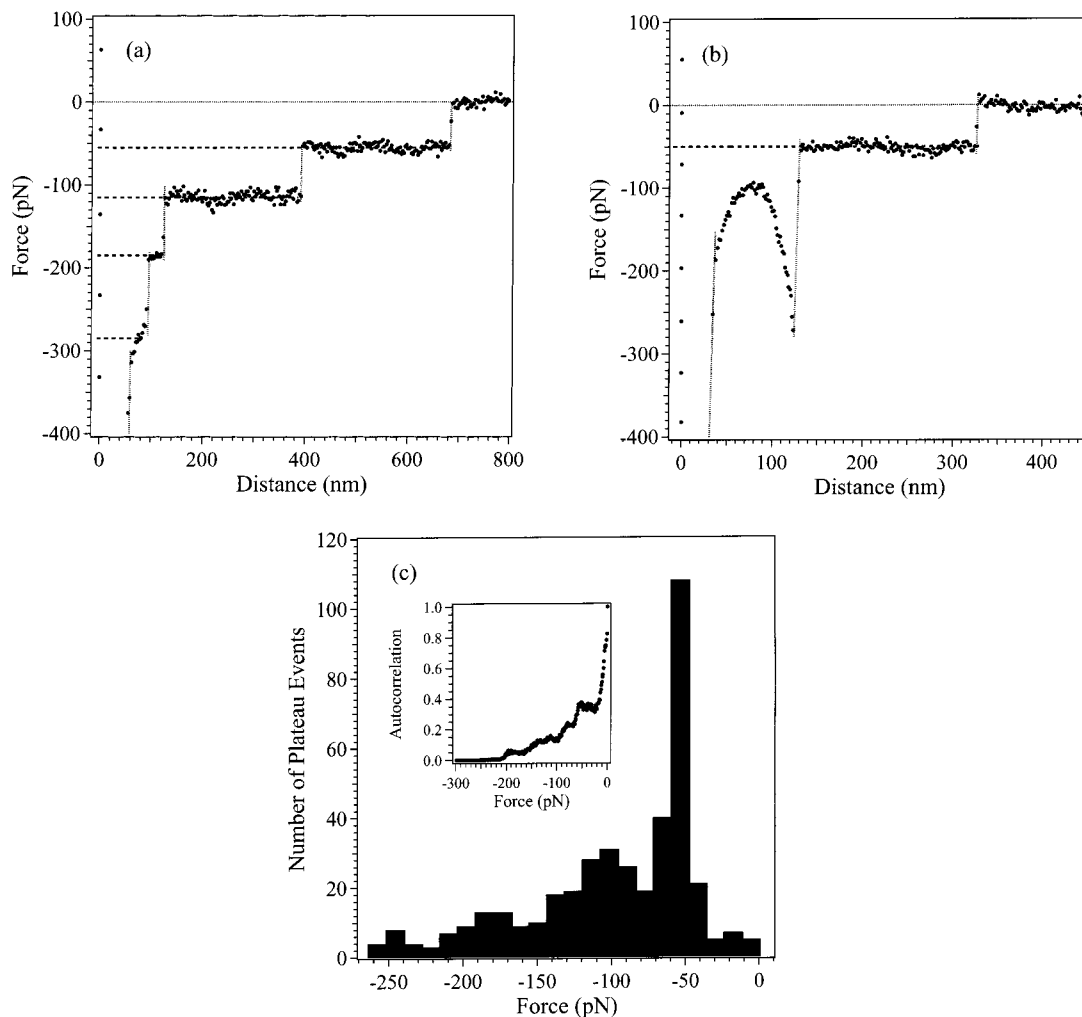


Figure 5. Representative force profiles and histogram summarizing the set of profiles for PEO in 0.45 M K_2SO_4 at 25 °C. The spring constant of the cantilever is 0.040 N m^{-1} , and the rms baseline noise is 22.0 pN. The discontinuities in both force profiles have been connected by gray lines of fixed slope. (a) A force versus distance profile shows 3, arguably 4, Plateau events each highlighted by the dashed lines. We have included the dashed line for the apparent Plateau event which is obscured by primary adhesion. The mean forces associated with each plateau are 285, 185, 115, and 55 pN. These plateaus persist to extension distances of 80, 120, 391, and 679 nm. (b) A force versus distance profile shows simultaneous Langevin and Plateau events. This corresponds to pulling two chains: a chain with two ends fixed to the surfaces, stretched and detached at 120 nm, and a chain with a free end which is pulled out of a surface globule at 330 nm. (c) A histogram shows the frequency of plateau forces. This histogram was constructed from 406 Plateau events in a data set of 600 force profiles. The magnitude of the plateau force is the mean of a Gaussian distribution, fitted to the experimental points of the plateau. The number of Plateau events in the data set peaks at nearly interval steps, suggesting that the plateaus are quantized according to the number of chains in the pulled polymer filament. The bin size of the histogram is 12.0 pN.

there is about 1/2 the data of Figure 5, it appears that the results are similar, suggesting the extension of single and double loops of PEO. The statistics of the PEO/ KNO_3 systems are less conclusive. One can argue that there are peaks in the histogram at 40 pN and 90 pN; however, the large rms error in the baseline of the AFM force data, conveyed in the larger bin size used in the histogram, makes the PEO/ KNO_3 system less conclusive.

At room temperature and in the absence of salts, the PEO force profiles do not contain Plateau events and consist entirely of Langevin events, verifying that good solvent conditions prevail. When the temperature is elevated to 30 °C or salt is added at room temperature, then we find Plateau events occurring with the same frequency as Langevin events. In the PNIPAM system, the onset of Plateau events occurred when the temperature was elevated just above the literature value of the LCST for bulk PNIPAM solution. However, in the case of no-salt aqueous solutions of PEO, the literature value of the LCST is 96 °C and much higher than our temperatures;

yet we find the onset of Plateau events as the temperature is elevated slightly from 25 to only 30 °C. This is also the case in the salt solutions. Bulk aqueous solutions of PEO in 0.45 M K_2SO_4 and 0.25 M KNO_3 have reported LCSTs of 34 and 65 °C, respectively. However, we find that the addition of these salts to aqueous solutions of PEO at room temperature is sufficient to cause the onset of these Plateau events. We can presume that the attractive silicon nitride surface plays a role in lowering the effective LCST of the PEO solutions. However, why the effective LCST of the PNIPAM solution is not lowered is not evident to us. When electrolyte is present, the local concentration of electrolyte is significantly larger at the silicon nitride surface than in the bulk due to cation adsorption at the anionic surface, and we can presume that this plays a role in further lowering the effective LCST of the PEO solution. But irrespective of how we describe bulk solution conditions, it is clear that with a slight elevation of temperature or the addition of salt, the pull-out of a free end now

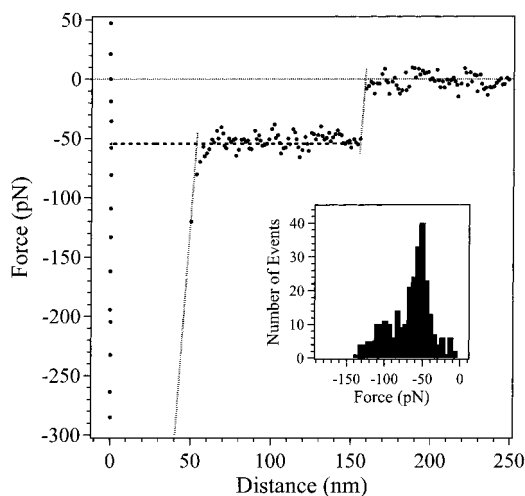


Figure 6. Force versus distance for PEO in aqueous no-salt solution at 30 °C. This is a typical force profile showing a single Plateau event. The discontinuities in the force profile have been connected by a dotted line and have a fixed slope taken to be the spring constant of 0.019 N m^{-1} . The baseline noise is 9.8 pN. The inset is a histogram of the frequency of Plateau events as a function of the magnitude of the plateau force, constructed from 280 Plateau events in 1500 force profiles. Plateaus of ≈ 55 pN are dominant, while there is arguably another smaller population of plateaus of ≈ 100 pN. The bin size is 5.4 pN.

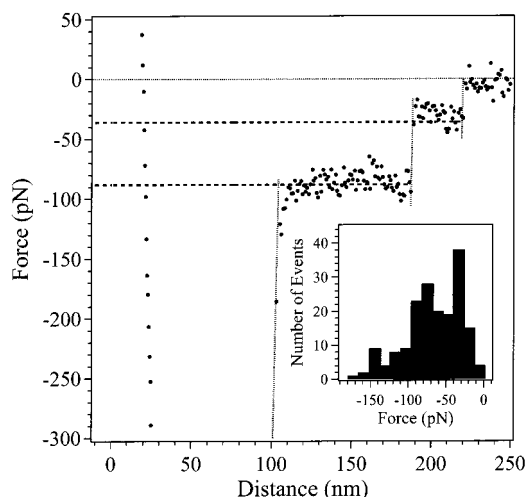


Figure 7. Force versus distance for PEO in aqueous 0.25 M KNO_3 solution at 22 °C. This is a typical force profile showing multiple Plateau events. The discontinuities in the force profile have been connected by a dotted line and have a fixed slope taken to be the spring constant of 0.062 N m^{-1} . Horizontal dashed lines in profile are used to highlight the constant force of the Plateau events. The rms baseline noise is 25 pN. The inset is a histogram of the frequency of Plateau events as a function of the magnitude of the plateau force, constructed from 180 Plateau events in 500 force profiles. The plateau forces range up to 150 pN; however, in comparison to the PEO/ K_2SO_4 system, it is difficult to argue that there are interval peaks in the histogram. The bin size is 13.8 pN.

requires energy. This energy is attributed to pulling monomer-by-monomer through a polymer-solvent interface.

V. Discussion

To summarize, we have shown that the extension of single PNIPAM and PEO chains in poor, aqueous solution occurs with a force that is independent of extension, i.e., a force plateau. The quantized steps associated with multiple plateaus in individual force profiles as well as

the statistics of a large collection of force profiles strongly suggest that these force plateaus are attributable to single chains. More generally, this force plateau can be understood in terms of the Rayleigh instability for a liquid column: extension of the chain results in monomer-by-monomer pull-out from the condensed globule into the poor solvent. That the plateau is a feature of the dominant surface tension, rather than adhesion of the chain to the substrate, is demonstrated by a number of observations. (1) The force of detachment in the Langevin events is 1 order of magnitude larger than the plateau force. That is, the force at which one end of the stretched chain is detached from the surface is much larger than the plateau forces. One might argue that elevated temperatures or the addition of salt might lower the affinity between the polymer and surface thereby reducing the force needed to detach a chain from the surface. However, we find that, under these conditions, both Langevin and Plateau events occur in the same force profile, the detachment force in the Langevin events remain at least 1 order of magnitude higher, and the surface affinity is not diminished. (2) The onset of Plateau events occurs generally with the onset of poor solvent conditions. In the case of PNIPAM, this is attained by elevating the temperature beyond the bulk LCST. However, in the case of PEO, the onset of plateaus occurs before the poor solvent conditions are achieved in the bulk. That is, only slight temperature elevations or salt additions is sufficient to see Plateau events. One might argue that bulk solvent conditions are not expected to accurately describe the polymer-solvent interactions near an adsorbing surface. However, polymer-solvent interactions near the interface will become more unfavorable with the addition of cosolvents/temperature change which bring the bulk solution closer to poor conditions. (3) Plateau events occur simultaneously with Langevin events in the same force profile. This suggests that the plateau force arises from the pull-out of a free chain end from a surface-bound globule while the Langevin event is associated with the comparably high stretching forces of a doubly end-tethered chain.

The aqueous PNIPAM and PEO/salt systems were studied previously by others using AFM and the surface force apparatus. Were force plateaus seen in these studies and, if not, why? In an early study, Braithwaite et al.^{31,32} used a colloidal sphere attached to the end of an AFM cantilever to probe PEO in aqueous KNO_3 using similar conditions to ours. Although their probe area is very large, plateaus that range between 750 and 3800 pN (converted from energy to force using the Derjaguin approximation³³) are evident in their published profiles. However, because the probe area is so large, it is unlikely that single chains are being pulled out, as also suggested by the large magnitude of the force. Nevertheless, the plateaus are evident. In another study, Oesterhelt et al.⁷ added cosolvents to aqueous PEO which was end-tethered to a substrate and probed with an AFM tip. The grafting density was not reported, but Langevin events were recorded at all reported solvent conditions, with fits to the force curves providing evidence of the solvency. No plateaus were reported. However, given that the chains were end-tethered to one surface, we would not expect to discern plateaus even if the grafting density were high. Because all chains were end-tethered to one surface, we would find surface-bound globules mostly on the grafting

(31) Braithwaite, G. J. C.; Howe, A.; Luckham, P. F. *Langmuir* **1996**, *12*, 4224.

(32) Braithwaite, G. J. C.; Luckham, P. F. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 1409.

(33) Derjaguin, B. V. *Kolloid-Z.* **1934**, *69*, 155.

surface and not on the AFM tip. Thus, pulling a chain monomer-by-monomer from globule through the polymer-solvent interface and into the solvent could only occur from globules on the grafting surface. A plateau should, under these circumstances occur, but because the chain is end-tethered, the plateau converts smoothly into a sawtooth stretching profile. The force profiles of Oesterhelt et al. could not contain a plateau with a discontinuous step. Identification of separate Plateau events and Langevin events would be nearly impossible, and the best one could do is note changes in the elasticity as evidence by the form of the force at high extension and just before detachment. More recently, Zhang et al.⁸ investigated the influence of a cosolvent and thermal treatment on PNIPAM using single chain extension with AFM. Their profiles contained exclusively Langevin events, and no plateaus were found. However, these authors have also communicated to us that poor solvent conditions were not tested and the solvent temperature was not maintained in situ or during the AFM pulling experiments.

A cantilever which is not sufficiently soft ($k \sim 0$) or stiff ($k \rightarrow \infty$) will provide a force profile which details the mechanical properties of both chain and cantilever, particularly at small length scales.³⁴ Thus, it is important to ascertain that the force profiles reported here are indeed representative of the chain and not the cantilever. Consider the case of an infinitely stiff cantilever where we have “magic” detectors for determining the force on the cantilever. This is a perfect, but unrealizable, AFM where the measured force is the chain tension as a function of the controlled extension, D . The partition function of the chain is $Z_0(D) = \int_0^\infty d\mathbf{x} \exp(-\beta U(\mathbf{x}))$, where $\beta = (k_B T)^{-1}$, $U(\mathbf{x})$ is the energy of the chain, and \mathbf{x} is the vector of coordinates of the chain which bridges the surface and cantilever tip. Distance D is the experimentally controlled variable, and because the cantilever is infinitely stiff, it is also equal to the extension of the chain. The Helmholtz free energy of the system (cantilever and chain) is that of the chain and is $-\beta^{-1} \ln Z_0$. The force exerted by the chain on the cantilever is

$$f_0(D) = \beta^{-1} \frac{\partial(\ln Z_0)}{\partial D} \quad (1)$$

To describe the forces of chain pull-out, we can express the energy of the chain at extension x as $U(x) = \Delta x$, where Δ is an energy per unit distance associated with pulling the chain monomer-by-monomer through the interface into poor solvent. The bare force of pull-out is then $f_0(D) = \Delta$, i.e., a plateau force.

However, a real AFM does not have a perfectly stiff cantilever. To increase sensitivity in the force measurement, it is most practical to have a soft or compliant cantilever, i.e., k as small as possible. The experimentally controlled distance D is then the chain extension and the cantilever deflection; the measured force will be the bare force, $f_0(D) = \Delta$, plus some correction term. How large is this correction term relative to Δ ? To determine this we need to consider the partition function of the entire system, chain plus cantilever. The energy of the cantilever is $1/2k(x - D)^2$, where x is the extension of the chain and the partition function of the system is then

$$Z(D) = \int_0^\infty dx \exp(-\beta \Delta x) \exp\left(-\frac{\beta k}{2}(x - D)^2\right) \quad (2)$$

The measured force is $f(D) = \beta^{-1}(\partial(\ln Z)/\partial D)$, or

$$f(D) = f_0(D) + \sqrt{\frac{2k}{\pi\beta}} \frac{\exp\left(-\frac{\beta}{2k}(kD - \Delta)^2\right)}{1 + \operatorname{erf}\left(\sqrt{\frac{2\beta}{k}}(kD - \Delta)\right)} \quad (3)$$

The second term on the rhs is the noise-dependent correction term. We can estimate the magnitude of this term and compare with $f_0(D) = \Delta$. The spring constant of our cantilevers is on the order of a few pN \AA^{-1} or $\sim 0.1 k_B T \text{\AA}^{-2}$, and the value of Δ or the force plateau that we measure is on the order of tens of piconewtons or $\sim 1 k_B T \text{\AA}^{-1}$. Thus, as long as $D \gg \Delta/k$ or the controlled distance is greater than tens of angstroms, then the correction term is negligible. As our plateaus persist over a few hundreds of nanometers, we can safely say that our plateau forces are representative of the chain pull-out. In addition, it is important to contrast our reported plateau forces with the limit of sensitivity of our AFM. Our AFM has a resolution limit of slightly under 10 piconewtons, but the Plateau events that we report are a few tens of piconewtons. Figure 3 shows that there are some Plateau events in the PNIPAM system which are recorded at or below this limit of 10 piconewtons. However, plateaus of 15 pN are most populous and the average plateau force is higher and easily detected by the AFM. The PEO systems show larger plateau forces of ~ 50 – 55 pN, irrespective of the added salt or elevated temperature, and these are well above the sensitivity limit of our AFM. However, in some measurements there is increased baseline noise which we attribute to scattering of the laser beam by PEO/salt.

There are two other explanations of force plateaus in single chain extension experiments: one based generally upon the rate of extension³⁵ and another based upon pulling a charged chain.^{9,36} Both treatments, however, predict force profiles with plateaus exclusively; i.e., Langevin and plateaus do not occur in the same force profile. In a generalized theoretical treatment, Haupt et al.³⁵ described the force profile of a single chain which adsorbs onto a surface in a series of loops. At extension rates which are faster than the rate of dissociation of the monomer-surface contact, the force profiles appears as a consecutive series of Langevin events, each corresponding to the stretching of an isolated loop of the chain. However, at slower extension rates where the monomer-surface contact has ample time to detach and re-form many times over the time scale of the pulling experiment, the force measured provides information about the strength of the contacts averaged over the length of the chain. Thus, for very slow extension rates, the force will be constant with smaller magnitude as the tension is distributed over all of the monomers in the chain. In the second explanation, Châtellier et al.⁹ describe the pulling of a single polyelectrolyte chain off an adsorbing, charged surface using scaling arguments. These researchers showed that the force profile reaches a plateau for extensions beyond the Debye screening length of the solution. The magnitude of the plateau force is indicative of the energy required to transfer monomers to the bulk solution from the electrical double layer near the surface. These explanations have been experimentally verified using AFM on polyelectrolytes adsorbed onto charged surfaces. Hugel et al.¹⁰ showed the dependence of the magnitude of the force plateau upon the polymer charge and electrolyte concentration. Previously, Châtellier et al.⁹ found plateaus when detaching

(34) Kreuzer, H. J.; Payne, S. H. *Phys. Rev. E* **2001**, *63*, 021906.

(35) Haupt, B. J.; Ennis, J.; Sevick, E. M. *Langmuir* **1999**, *15*, 3886.

(36) Châtellier, X.; Joanny, J.-F. *Phys. Rev. E* **1998**, *57*, 6923.

polyelectrolytes from charged surfaces. In both of these experimental verifications, plateaus were observed exclusively. The profiles which we presented in this paper are significantly different. First, our force profiles contain either exclusively Langevin events or Langevin and Plateau events, depending upon temperature and/or cosolute addition. Second, our profiles did not vary in character with rate of retraction of the AFM tip. Finally, we have neutral polymers. Nevertheless, the underlying physics of constant force plateaus is similar: plateaus signify the pulling of a chain monomer-by-monomer from an attractive potential into a zone of zero mean potential. In the Hugel and Châtelier experiments, this potential arises from the electrical double layer. In the experiments

that we report here, the potential arises from the local solvency of the chains.

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