

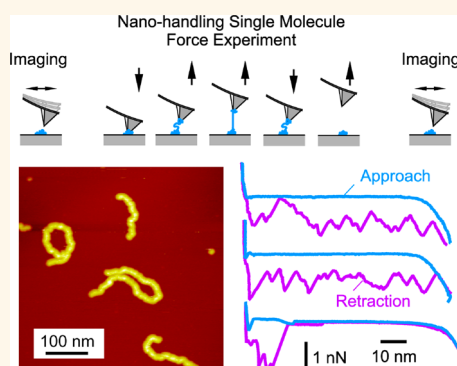
Single-Molecule Force Measurements by Nano-Handling of Individual Dendronized Polymers

Lucie Grebikova,[†] Plinio Maroni,[†] Baozhong Zhang,[‡] A. Dieter Schlüter,[‡] and Michal Borkovec^{†,*}

[†]Department of Inorganic and Analytical Chemistry, University of Geneva, Sciences II, 30 Quai Ernest-Ansermet, 1205 Geneva, Switzerland, and [‡]Department of Materials, Institute of Polymers, Swiss Federal Institute of Technology, ETH Zurich, HCI J 541, 8093 Zurich, Switzerland

ABSTRACT A nano-handling technique based on atomic force microscopy (AFM) is presented that allows reliable measuring of force–extension profiles of single-polymer molecules. The basis of the method is a properly functionalized solid substrate, to which a small amount of the polymers in question is adsorbed. The sample is first imaged in amplitude modulation mode in solution with a functionalized AFM cantilever, and a polymer chain is picked up with the AFM tip at one of the ends of the polymer molecule. Force curves are recorded by stretching the attached polymer molecule many times. After the force experiments, the molecule is imaged again. In this fashion, one can ascertain that the force experiments are truly carried out with one individual molecule. With this technique, the force response of amino-functionalized dendronized polymers was studied and it could be accurately described by the freely jointed chain model with chain elasticity.

A monotonic dependence of the mechanical properties of these polymers up to generation 4 was found. The elastic constant was independent of the generation and solution composition. On the other hand, the effective Kuhn length increased with the generation at higher salt concentrations. The mechanical response of dendronized polymers can be tuned with solution composition.



KEYWORDS: single molecule · dendronized polymers · dendron · force spectroscopy · scanning probe microscopy · atomic force microscopy

For about two decades, we have witnessed systematic studies of the mechanical response of individual polymers on the single-molecule level. Individual DNA molecules were first studied with magnetic tweezers.^{1–3} Soon afterward, it was realized that the atomic force microscope (AFM) represents an ideal tool to investigate in a similar way smaller macromolecules, particularly, muscle proteins or polysaccharides.^{4–13} The AFM was later used to probe mechanical properties of synthetic polymers and polyelectrolytes.^{8,14,15} More recently, the mechanical response of bottle-brush and dendronized polymers (DPs) was studied with the AFM, as well.^{16–18}

The technique based on the AFM is also referred to as single-molecule pulling or force spectroscopy, and it has revealed substantial details concerning the mechanics of individual macromolecules. Such experiments were performed on proteins, and they revealed that stretching of such

protein proceeds through unwrapping of individual protein domains.^{5,12,19} For polysaccharides, it was established that they may undergo a conformational transition upon stretching.^{5,6,10,13} Similar conformational transitions were also found for poly(ethylene oxide).¹⁴ Interesting reports exist concerning possible modification of mechanical properties of individual polymers by external parameters such as optical excitation, redox properties, or nature of the solvent.^{14,17,20,21}

Investigations of mechanical properties of single polymers with the AFM rely on repeated approach and retraction force measurements, whereby the retraction part normally reveals the force response of single-polymer molecules. These studies are based on spontaneous bridging between an appropriately functionalized AFM tip and substrate with a polymer chain.^{5–8,14–19,22,23} The polymer can be either adsorbed or grafted to the substrate, but in such situations,

* Address correspondence to michal.borkovec@unige.ch.

Received for review October 21, 2013 and accepted February 8, 2014.

Published online February 08, 2014
10.1021/nn405485h

© 2014 American Chemical Society

several chains can be picked up simultaneously. In some cases, the polymer can also be attached to the sharp AFM tip, thereby improving the chances of probing single molecules.^{17,24,25} The approach part of the force curve may also reveal the force response of the polymer, which becomes possible when the tip is not retracted too far away from the surface, and the chain can be stretched several times.^{8,12–14,16,18} This approach can also be used to probe the conformational dynamics of macromolecules.^{12,13}

While the aim of such experiments is to investigate single-polymer molecules, the verification that just a single-polymer molecule is bridging the tip and the substrate is not trivial. This problem was normally resolved by plotting all force profiles *versus* a relative extension, whereby the latter is normalized at a given force.^{8,10,22,23} When one deals with single molecules, all force curves collapse on a single master curve. Other possibilities to ensure that single-molecule events are recorded include grafting of polymer molecules to the tip and not to the substrate and by exploiting the number of events recorded during a retraction.^{8,15,22} In the former case, the probability that a single molecule is being picked up from a tip is larger than when the molecules are being picked up from a planar substrate. In the latter case, one event in the retract part suggests the involvement of a single molecule, while multiple events point toward several molecules, formation of loops originating of the same molecule, or their combination.¹⁵ Nevertheless, when these techniques are being used jointly, one can be rather confident to deal with single molecules. However, neither of these techniques provides direct evidence.

New generation low-noise AFMs combined with ultrasmall cantilevers with high resonance frequencies now allow high-resolution imaging and force measurements on the same sample in liquids.^{26,27} With such instruments, one can directly verify that a single-polymer molecule is involved by direct imaging. We have recently realized a similar protocol in a study of desorption of DPs from surfaces by peeling with appropriately functionalized AFM tips and by imaging the individual adsorbed polymers before and after the force experiment.²⁷ A similar approach was used earlier to investigate the mechanical response of individual proteins that were lifted up from a compact membrane and imaged in the contact mode.^{28,29}

In the present article, we present a novel nano-handling technique that combines imaging and force measurements with a single-polymer molecule. This technique relies on attaching a polymer molecule to the AFM tip and recording force curves with the same molecule numerous times. The fact that one deals with a single-polymer molecule is ascertained by AFM imaging in the amplitude modulation mode of the adsorbed molecule before and after the force experiment with the functionalized AFM tip. This tool is used

here to study the mechanical response of amino-functionalized DPs of different generations and at different salt levels. While the same system was studied with the conventional pulling technique earlier,¹⁷ the present technique reveals that the conventional technique sometimes does not always provide information at the single-molecule level.

RESULTS AND DISCUSSION

Mechanical properties of individual adsorbed polymer molecules were measured with the single-molecule nano-handling technique. This approach operates truly on the single-molecule level, and the response of the same molecule can be probed many times. The experiments were carried out with amino-functionalized DPs. The structure of these polymers is illustrated in Figure 1. To each monomeric unit, a dendron of a given generation is attached as a side chain. Generations 1–5 were studied. Overview AFM images obtained with mixtures of three different generations indicate the flexible nature of these polymers. Cross-sectional dimensions increase with the generation from about 2 nm for PG1 to about 10 nm for PG5. Generations up to PG4 feature a uniform cylindrical envelope of the adsorbed chain, while for PG5, a characteristic pearl necklace structure can be seen. More details on the dimensions and characteristics of the adsorbed chains can be found elsewhere.^{27,30}

Substrates. The success of the nano-handling experiments relies on the appropriate adhesion properties of the surface, which must be adjusted for a given type of polymer and solution condition. When the molecules bind too weakly to the surface, only peeling events are observed. If they are attached too strongly, the

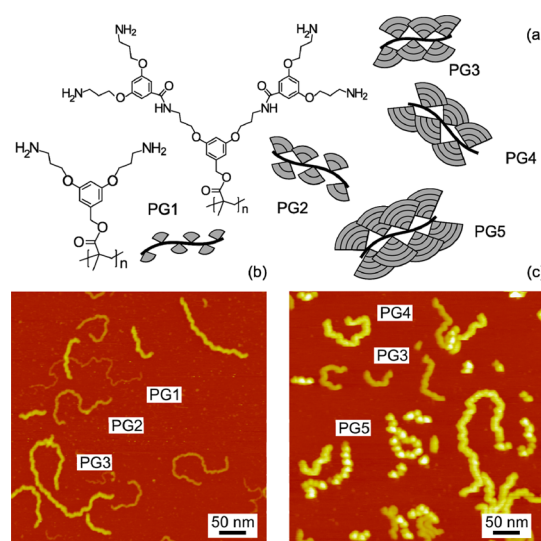


Figure 1. Dendronized polymers of different generations used in this study. (a) Schemes and chemical structure of the dendronized polymers. AFM images of an electrolyte solution of pH 4.0 and an ionic strength of 0.1 mM for (b) PG1, PG2, and PG3 and (c) PG3, PG4, and PG5.

TABLE 1. Characteristics of the Surfaces Used

surface	time ^a	rms roughness ^b (nm)	contact angle ^c (deg)
silica		0.21	38.2
EF-silica	30 min	0.23	51.2
AF-mica	45 min	0.09	55.5
AF-mica	90 min	0.10	57.1
AF-mica	12 h	0.12	67.5

^aTime of functionalization. ^bMeasured by AFM in solution. ^cAverage of several replicate measurements in air.

molecule cannot be picked up. Optimal conditions to observe pulling events can be found between. These conditions could be realized for the DPs investigated here by vacuum silanization of the silica or mica surfaces as summarized in Table 1.

DPs adhere relatively weakly to bare silica. Silica, therefore, has to be made more adhesive, which was achieved by epoxide functionalization (EF-silica). On the other hand, DPs adhere to bare mica strongly. This substrate thus has to be made less adhesive by amino functionalization (AF-mica). The silanization makes the surfaces more hydrophobic, as illustrated by the corresponding increase of the contact angle (Table 1). With increasing silanization time, the roughness does increase somewhat, too. The chosen reaction time is important since longer times promote weaker adsorption, leading to more peeling and less pulling events.

Different surfaces were used to obtain optimal conditions for pulling experiments. These conditions depend on the DP generation and on the composition of the electrolyte solution in which the experiments are being carried out. DPs stick to surfaces better with increasing generation and increasing salt level.²⁷ Bare silica or EF-silica was suitable for all DPs at low salt concentration. At higher salt concentration, AF-mica was found to be more appropriate. Thereby, shorter silanization times were chosen for the lower generations and longer ones for higher generations. The actual surfaces used are summarized in Table 2.

In some cases, different substrates could be used to obtain good conditions for pulling. This feature was used to verify that the nature of the substrate did not influence the mechanical properties of the DPs. The results obtained with two different substrates were compared, and the same mechanical properties were always found for the same DP generation and solution composition.

Single-Molecule Nano-Handling Technique. This technique proposed here enables one to measure numerous force–extension and retraction curves with a single molecule. Its principle is illustrated in Figure 2 (top). The DPs are adsorbed from a dilute DP solution of a concentration of 4–6 mg/L to an appropriately functionalized substrate. The substrate is then incubated in the electrolyte solution and imaged with the AFM with a functionalized tip to locate the target molecule. The tip

TABLE 2. Surfaces Used for Different Experiments

generation	salt concentration (mM)	surface ^a	time ^b
PG1	0.1	EF-silica	30 min
	100	AF-mica	45 min
PG2	0.1	EF-silica	30 min
	100	bare silica	
PG3	0.1	EF-silica	30 min
	100	AF-mica	45 min
PG4	0.1	bare silica	
	100	AF-mica	12 h
PG5	0.1	EF-silica	30 min
	100	AF-mica	90 min

^aSurfaces used for adsorption. ^bTime of functionalization.

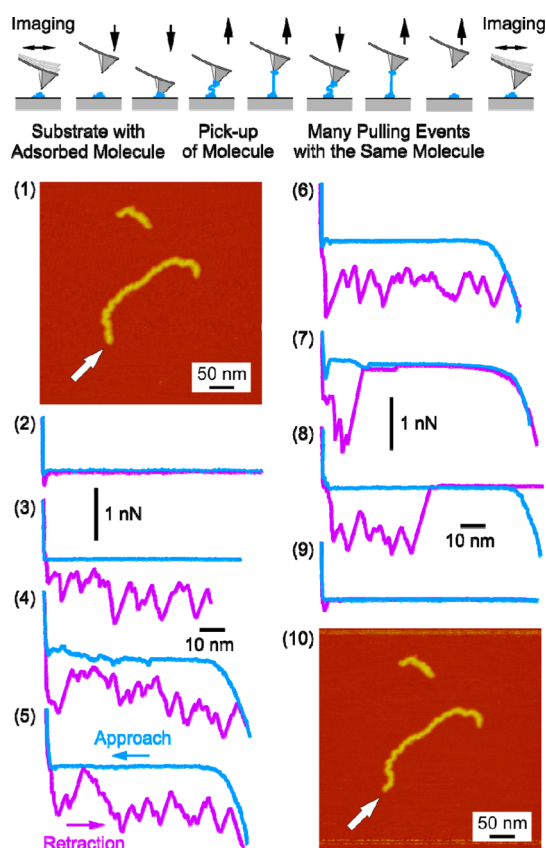


Figure 2. Principle of single-molecule nano-handling force experiments. The top scheme illustrates the principle of the experiment. An adsorbed molecule is first imaged in amplitude modulation mode. Subsequently, the molecule is picked up by the functionalized tip, and a series of force–extension profiles are recorded with the same molecule. Finally, the molecule is imaged again. Below, a realization for PG4 at pH 4.0 and an ionic strength of 100 mM on AF-mica silanized for 12 h. AFM images of the molecule investigated before (1) and after the force experiment (10). The arrow indicates the location of the force experiment. A series of approach and retraction force curves are shown between (2–9). The force–extension curves of the molecule can be best observed in the approach part of the curves (4–9) but sometimes also upon retraction (7). A displacement of the lower quarter of the molecule during the experiment can be seen in the AFM images.

is then placed over the end of this molecule, and a series of vertical approach and retraction cycles are initiated. Once the molecule is picked up, it can be stretched and released many times. At one point, the molecule detaches again from the tip and readsorbs to the surface again. The readsorbed molecule is imaged, too.

Figure 2 (bottom) shows the raw data for a typical realization of such an experiment for PG4. Polymers are adsorbed from an electrolyte solution of pH 4.0 and an ionic strength of 100 mM to AF-mica. The target molecule is shown in the AFM image (1). The tip is centered over the lower end of the molecule (arrow), and a series of the force–distance approach–retraction curves are recorded (2–9). During the first approach curve (2), the molecule is not picked up by the tip. In the second attempt (3), the molecule is being lifted up, as evidenced by the sawtooth-like retraction profile indicating various desorption steps. The molecule now bridges the tip and the substrate. During the next cycle (4), the lifted molecule exerts a force on the tip, which is released during approach. The molecule readsorbs but can be lifted up again by retracting the tip. The following cycles (5–8) show clean force–extension curves of the molecule upon approach. The molecule adsorbs more weakly with progressive lifting, and cycle (7) shows evidence of force–extension curves of the molecule upon approach and retraction. During the next attempt (8), the molecule desorbs from the tip, as evidenced by the next cycle (9). The molecule is then imaged again (10). One observes that about the lower quarter of the molecule was displaced, which roughly corresponds to the contour length suggested by the force–extension curve.

Among the adsorbed molecules, typically every fourth to 10th molecule can be picked up. Suitable molecules can be lifted quickly within the first few approach–retraction cycles, and then they can be normally stretched 3–10 times before they detach from the tip. In some cases, the same molecule can be picked up again, but at one point, it is no longer possible to lift it up. In that case, one looks for another suitable molecule on the surface. Dwelling with the tip on the molecule during a few seconds did not increase the likelihood to pick up a molecule substantially. The approach curves are often more suitable than the retraction curves for recording the force response of single molecules because the molecules may interact with the substrate during retraction. For lower generations or after several lift-ups of the molecule, however, such interactions are weaker, and in these situations, the retraction and approach curves can both be used. Since the nano-handling technique targets specific molecules and records numerous force profiles with many of them, the overall probability to record a pulling event is very high, typically 0.1–0.3. This high probability of pulling events represents a substantial advantage over the classical pulling technique, where

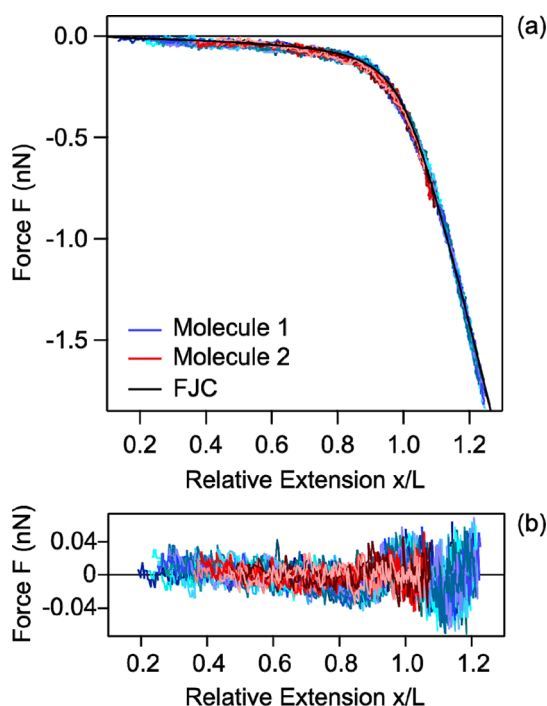


Figure 3. Force versus relative extension of different PG2 molecules of an electrolyte solution of pH 4.0 and an ionic strength of 0.1 mM on EF-silica and together with best fit of the freely jointed chain model. (a) Force curves and (b) corresponding residuals. Both molecules show the same response within experimental error.

often hundreds of attempts are needed to encounter a successful event.

Single-Molecule Force Response. This nano-handling technique enables us to collect numerous force–extension relationships for exactly the same polymer over a wide force range, typically 0.01–1 nN. The technique further ensures that the measurements are collected with a single molecule because the molecule is imaged before and after the force experiments.

Figure 3a shows a series of force curves recorded with two different PG2 molecules in an electrolyte solution with an ionic strength of 0.1 mM adsorbed to EF-silica. For the first molecule, 31 force curves were measured and 25 for the second one. The data were rationalized in terms of the freely jointed chain (FJC) model including the elastic response. The magnitude of the applied force F and the extension x follows the relationship¹⁴

$$\frac{x}{L} = \coth\left(\frac{lF}{kT}\right) - \frac{kT}{lF} + \frac{F}{K}$$

where L is the contour length, l the effective Kuhn length of a segment, k the Boltzmann constant, T the absolute temperature, and K the elasticity constant. The contour length can be different from one curve to another since during the retraction the polymer can be further detached from the substrate. We have therefore initially fitted each curve individually by further allowing for a shift in the baseline because a perfect

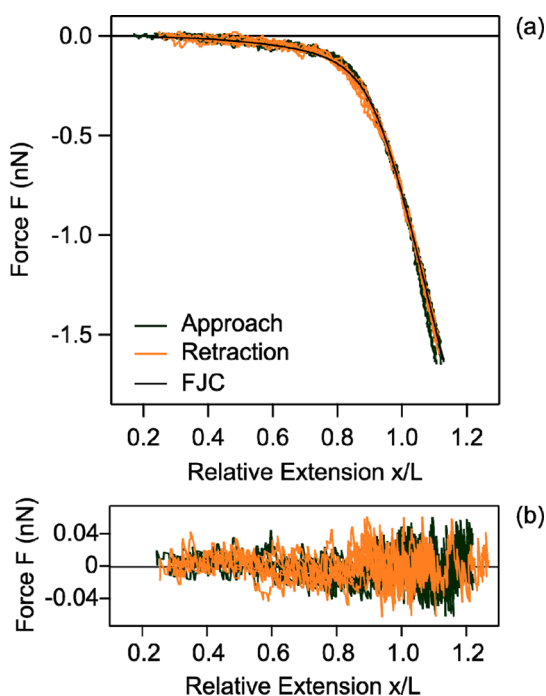


Figure 4. Force versus relative extension of a PG2 molecule recorded upon approach and retraction of an electrolyte solution of pH 4.0 and an ionic strength of 0.1 mM on EF-silica together with best fit of the freely jointed chain model. (a) Force curves and (b) corresponding residuals. Approach and retraction lead to the same response within experimental error.

constancy of this baseline between different cycles cannot be assured within the force resolution achieved.

The resulting elasticity constants and effective Kuhn lengths were subsequently averaged, and the fit was repeated by fixing these two parameters to their average values but still allowing for different contour lengths and baseline shifts. Figure 3 summarizes final results of this global fit of the force profiles. They are normalized to the relative extension, and the baseline shift has been subtracted. One obtains an elasticity constant of $K = 6.7 \pm 0.9$ nN and effective Kuhn length of $l = 0.22 \pm 0.03$ nm. The residuals shown indicate that the fit is very accurate and features a standard deviation of about 0.017 nN. This number is somewhat larger but well comparable to the noise in the force curves of about 0.008 nN.

Figure 4 compares force profiles recorded during approach and retraction for one particular PG2 molecule in an electrolyte solution with an ionic strength of 0.1 mM adsorbed to EF-silica. The profile can be again well-fitted with the FJC model leading to the elastic constant $K = 6.8 \pm 0.8$ nN and an effective Kuhn length of $l = 0.17 \pm 0.04$ nm. These values are within the error bar of the ones determined for the two different molecules shown in Figure 3. This comparison reveals that the approach and retraction force curves are identical within experimental error. This point indicates that the extension process is fully reversible

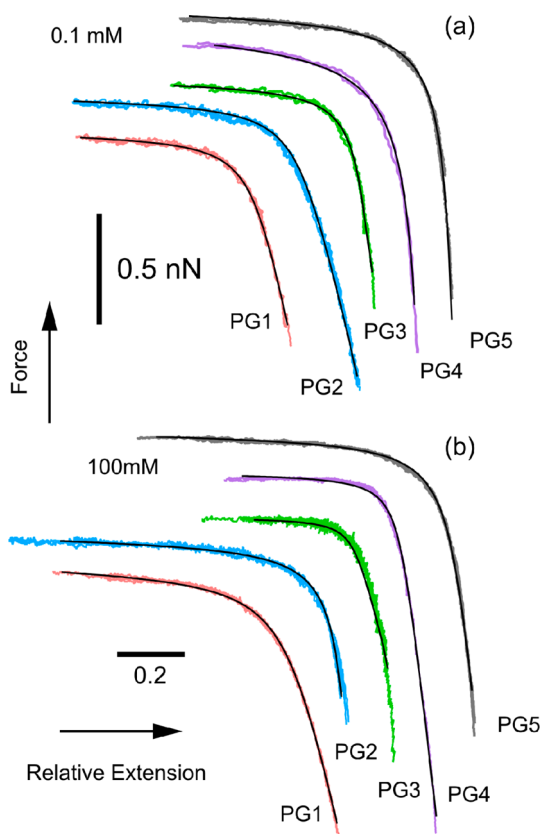


Figure 5. Force versus relative extension of different generations of dendronized polymers together with best fit of the freely jointed chain model. The measurements were carried out at pH 4.0 at an ionic strength of (a) 0.1 mM and (b) 100 mM. The curves have been displaced for clarity. The substrates used are summarized in Table 2.

on the time scale of the experiment, which typically is around 0.1 s.

The force profiles were further measured for different generations from PG1 to PG5 and for two different ionic strengths of 0.1 and 100 mM. The results are shown together with the best fits of the FJC model in Figure 5. One observes that the model provides an excellent description of all force curves over the entire force range within the experimental noise. The only exception is PG5, where the curvature of the force profile is not described accurately, but the deviation cannot really be seen on the scale of the figure. We found that this force profile could be better described with the worm-like chain model.² However, the differences were minor, and this aspect was not pursued further.

Let us now discuss the dependence of the fitted parameters on the generation and the solution composition. Figure 6 summarizes these parameters versus the generation of the DPs for the two ionic strengths investigated. One observes that PG5 behaves differently from the DPs of lower generations. For the lower generation polymers up to PG4, the elasticity constant is $K = 12 \pm 4$ nN. This value is independent of the generation and the salt level within experimental error.

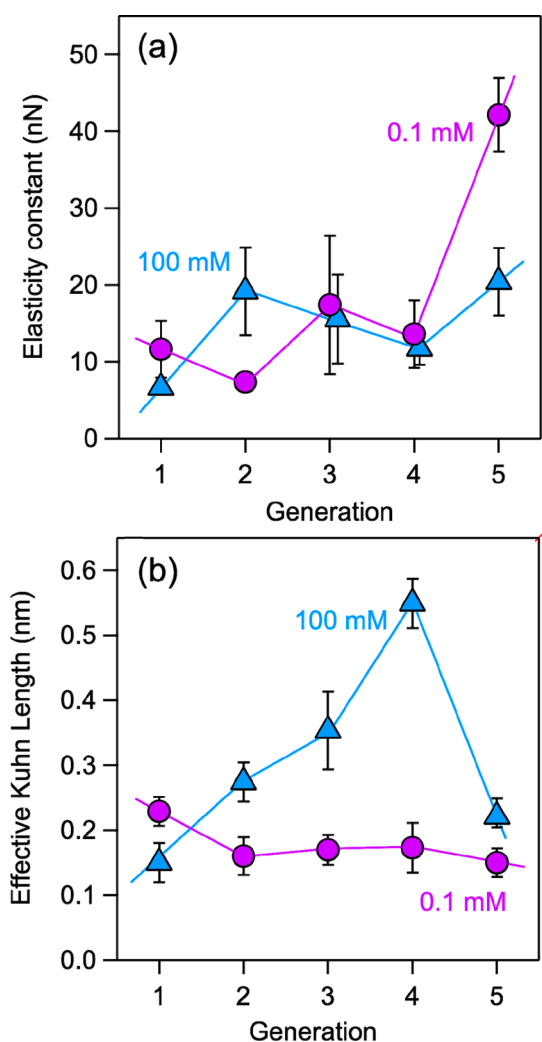


Figure 6. Generation dependence of the best fit parameters of the force–extension curves with the freely jointed chain model at two different salt concentrations. (a) Elastic constant and (b) effective Kuhn length. The substrates used are summarized in Table 2.

The effective Kuhn length is also independent of the generation, but only at low salt levels, where it has the value $l = 0.18 \pm 0.03$ nm. However, at higher salt levels, its value increases monotonically with the generation, whereby it starts at about the same value for PG1 and attains $l = 0.55 \pm 0.04$ nm for PG4. This dependence of the force response on the salt level for PG4 can also be observed in Figure 5. The force curves show a shallow transition from the low to high force regime at low salt concentrations, while at higher salt levels, this transition becomes increasingly sharper with increasing generation.

The fact that the elasticity constant shown in Figure 6a is independent of the generation could be related to the fact that the stretching response of the DPs is dictated by the poly(methyl methacrylate) backbone and thus independent of the size of the dendrons. This point of view is supported by the fact that the value reported here is comparable to the

elasticity constant of the alkane backbone.³¹ On the other hand, the effective Kuhn length of a DP increases with increasing salt concentration, especially for PG3 and PG4, as illustrated in Figure 6b. This behavior is at odds with the argument that electrostatic interactions should lead to a decrease of the persistence length and an increased flexibility at higher salt levels.²² The observed trend may thus be related to the hydrophobicity of the polymer backbone. Since the volume of the hydrophobic region increases with increasing generation and the charged hydrophilic ammonium groups are screened with increasing salt level, one expects a strengthening of the hydrophobic interactions within the DP. The hydrophobic interactions probably overrule the electrostatic effect. A similar but weaker increase of the effective Kuhn length with the salt concentration was equally reported for poly(vinyl amine).²³ This effect could also be a manifestation of an analogous mechanism involving hydrophobic interactions. Another explanation might involve the coupling between the stretching response and the ionization equilibrium, which may also induce a nonmonotonic dependence on the salt concentration.²⁴

The mechanical response of PG5 is different from all lower generations. The effective Kuhn length does not show any dependence on the salt level and is close to the value quoted above. On the other hand, the elastic constant lies clearly above the values measured for the other generations, especially at low salt levels, where $K = 42 \pm 5$ nN. These differences are likely to be related to the pearl necklace structure of the PG5 polymer. However, a more detailed interpretation of these results seems premature at this point.

The effective Kuhn lengths reported here are almost 1 order of magnitude smaller than the persistence lengths obtained from AFM imaging of similar DPs.³² Analogous discrepancies were reported between Kuhn or persistence lengths obtained from single-molecule force experiments and scattering experiments.^{22,23,33} These discrepancies are rationalized by observing that the force experiments probe the bare persistence length while the persistence length determined by other techniques also includes the electrostatic part.³⁴

Comparison with Classical Pulling Experiments. Mechanical properties of the same DPs were investigated earlier with the classical pulling technique.¹⁷ While the effective Kuhn lengths reported in the latter reference are in good agreement with the ones observed here, the elasticity constants were claimed to decrease with increasing salt concentration. This trend cannot be confirmed with the current approach at the single-molecule level. We suspect that these discrepancies originate from contributions from desorption DPs off the surface or from bundle formation. Desorption of the DPs could occur while peeling the polymers off the

substrates since there was no covalent attachment between the molecules and the substrate. Formation of bundles of DPs is another possibility, as the force response of a bundle of DPs would be different than that of a single chain. The formation of such bundles in solution was confirmed by electron microscopy.^{35,36} Individual polymer chains could also slide past each other within such bundles upon pulling. Nevertheless, the present work confirms the main conclusion of the previous study that the mechanical response of these polymers can be tuned by the salt level.

CONCLUSION

We present a novel AFM nano-handling technique that is capable of accurately measuring force–extension relations of individual polymer molecules. Thereby, a previously adsorbed polymer chain is picked up with the AFM cantilever, and the force curves are recorded by stretching an individual polymer molecule many times. The fact that one deals with single, individual molecules is verified by AFM imaging of the polymer chain in question before and after the nano-handling experiment. Such experiments can be easily repeated for different molecules from the same sample, whereby any eventual heterogeneities within the sample can be detected.

This technique was used to study the force response of amino-functionalized DPs. This response could be accurately described with the freely jointed chain model including a linear elasticity term over a wide force range of 0.01–1 nN. We found monotonic dependence of the mechanical properties of these polymers up to generation 4. The elastic constant was found to be independent of the generation and solution composition. However, the effective Kuhn length did increase with the generation at high salt concentrations, while it remains constant at low salt levels. We suspect that this trend is related to the modification of the hydrophobic character of the polymers in the presence of salt. The mechanical response of PG5 is different, most probably due to their pearl necklace structure.

The main advantage of the present nano-handling approach over the conventional pulling technique is

that mechanical properties of the polymer molecules can be studied truly on the single-molecule level, as confirmed by imaging directly before and after the force experiments. The classical pulling technique uses less direct ways to ensure that single molecules are being probed, mainly by investigating the scaling properties of the force curves or by exploiting the number of pulling events within a force curve.^{8,22} Another advantage of the nano-handling technique over the conventional one is that the probability that a polymer chain is picked up is large, and therefore, a substantial number of force curves can be easily analyzed. The probability to record a pulling event with the conventional technique is often minute, and as a consequence, a large number of approach and retraction cycles must be recorded. The nano-handling technique is therefore much more efficient, and one can easily collect a large number of force curves, leading to more accurate results with good statistics.

The present nano-handling technique further offers new opportunities to explore mechanical properties of individual polymer chains. One possible direction of investigation is to specifically address influence of a particular position along the polymer chain on the mechanical response. One may lift up the chain at its end, as was done here. Alternatively, one should be able to lift the molecule in the middle and thus explore the response of a loop. Another use of our technique would be to carry out more extensive nanomanipulation to obtain a particular arrangement of molecules on a surface. For example, one may pick up a chain, stretch it, deposit the chain in its stretched state, and subsequently compare its response with a nonstretched one. Alternatively, one could position two molecules at the same location and explore the mutual influence on their mechanical response depending upon the aggregate structure of the molecule (e.g., parallel *versus* helix). We suspect that these exciting possibilities will be exploited soon, and therefore, the present nano-handling technique is likely to become quickly popular in the AFM research community.

EXPERIMENTAL SECTION

Dendronized Polymers. Polymethacrylate-based DPs of different generations (PG n , $n = 1–5$) terminated with amine groups were synthesized with the attach-to route as described elsewhere.^{32,37} The side dendrons have a polydispersity index of about 1.01 with respect to the expected number of the amine groups for each repeat unit. DPs were dissolved in electrolyte solutions adjusted to pH 4.0 with HCl. Under these conditions, the amine groups are positively charged. Further details concerning the characterization of these DPs by gel permeation chromatography and AFM are given elsewhere.^{27,32,37}

Substrates. Three types of surfaces were used for the experiments, namely, bare silica, ethoxy-functionalized silica

(EF-silica), amino-functionalized mica (AF-mica). As silica surfaces, the native oxidized silicon wafer surfaces (Silchem, Germany) were used. The surfaces were cleaned for 20 min with hot piranha solution, which is a mixture of 98% H₂SO₄ and 30% H₂O₂ in a volumetric ratio of 3:1. Subsequently, they were washed with pure water, dried in a stream of nitrogen, and kept in water prior to use. EF-silica surface was obtained by vacuum silanization. Thereby, freshly cleaned silica surface was placed in an evacuated glass desiccator aside a 150 μ L drop of (3-glycidioxypropyl)-dimethylethoxy silane (Sigma Aldrich, Switzerland) for 30 min. High-grade mica was obtained from Plano (Wetzlar, Germany) and was cleaved in air immediately prior to use with scotch tape. AF-mica surface was obtained by vacuum silanization with 30 μ L of 3-(ethoxydimethylsilyl)propylamine (Sigma Aldrich,

Switzerland) for a specified amount of time that must be respected to obtain reproducible results. Static contact angles of these substrates were measured with a home-built setup. A drop of about 10 μL of an aqueous electrolyte solution of pH 4.0 and an ionic strength of 0.1 mM was placed on the substrate, and a magnified digital picture was taken. The contact angle was determined from an average of 3–5 images.

AFM Imaging. Images were recorded in electrolyte solutions with the Cypher AFM (Asylum Research, Santa Barbara, CA) in the amplitude modulation mode. Biolever mini cantilevers with silicon nitride tips (BL-AC40TS, Olympus, Japan) were used. These tips had a nominal tip radius below 10 nm and resonance frequencies around 30 kHz in water and around 130 kHz in air. The spring constants were in the range of 0.07–0.15 N/m as measured through thermal fluctuations in air. Substrates were prepared by adsorption from dilute DP solutions at concentrations of 4–6 mg/L for 40 s. This concentration was chosen to obtain a sufficient coverage of DPs on the surface, but such that only few overlapping chains would result. Subsequently, the sample was rinsed with water and dried with nitrogen. The samples were then incubated in the same electrolyte solution, and the images were acquired with a scan rate of 4.9 Hz, free oscillation amplitude (FOA) of about 20 nm, and a set point corresponding to around 70% of the FOA. Overview images were acquired with DP adsorbed from solutions where different generations were mixed at suitable concentrations. The images were similar for different types of substrates. Roughness of the functionalized substrates was obtained from AFM images of $1\ \mu\text{m} \times 1\ \mu\text{m}$ in liquid at pH 4.0 and an ionic strength of 0.1 mM. All AFM experiments were carried out at room temperature of $24 \pm 1\ ^\circ\text{C}$.

Single-Molecule Force Experiments by Nano-Handling. Prior to use, cantilevers were cleaned with a UV-ozone cleaner (PSD Pro, Novascan, Ames, IA, USA) in an oxygen-enriched atmosphere for 20 min and silanized overnight with (3-glycidioxypropyl)-dimethylethoxysilane as described above. The DPs were adsorbed from solutions with a polymer concentration of 4–6 mg/L for 40 s. The substrates were dried, mounted in the AFM, and incubated in electrolyte solutions of pH 4.0, where the ionic strength was eventually adjusted with KCl. At the start of each experiment, images were acquired in the electrolyte solutions in the same way as described above. The lateral resolution of the images obtained with the functionalized tips is somewhat inferior to the ones acquired with nonfunctionalized ones. To minimize thermal drifts during the force measurements, the DPs were first continuously imaged with the AFM for about 1 h. After stabilization, the piezo-excitation of the cantilever was stopped and the tip was positioned near the end of a selected molecule. Subsequently, vertical approach–retraction force cycles were recorded with a sampling rate of 2 kHz. To minimize the wear of the functionalized tip, a trigger deflection point $<30\ \text{nm}$ was used. The piezo-displacement and cantilever deflection were converted to force–distance curves. The standard deviation of the noise in the force curves was about 0.008 nN. Since the length of the investigated molecule could be estimated from its image, the pull-off distance was set to about one-half of this value. In this fashion, it was possible to record a series of force curves with the same molecule. After the force measurements, the same region was imaged again in order to observe the displacement of the targeted molecule. The investigation of a single molecule typically took 30 min to 1 h. The same experiments were carried out with other adsorbed molecules. After a few hours, however, functionalization of the tip ages and molecules cannot be picked up any longer. At that point, the cantilever must be cleaned and functionalized again. The cleanliness of the tip was checked by recording force curves in a part of the surface that was free of adsorbed molecules. A clean tip did not show any single-molecule events. Intermediate drying of the sample had no effect on the results, as was verified in a few cases by keeping the sample in the wet state. However, the procedure involving intermediate drying was more practical since the mounting of a dry substrate in the AFM is much simpler than of a wet one. The approach and retraction velocity used was normally 200 nm/s. Force–extension curves remained very similar when the velocity was decreased to 20 nm/s. Small differences in the force curves were observed at velocities exceeding 500 nm/s.

Conflict of Interest: The authors declare no competing financial interest.

Acknowledgment. Support of this research was provided by the National Research Program “Smart Materials” (NRP 62) and other programs of the Swiss National Science Foundation, COST Action CM1101, ETH Zürich, and University of Geneva.

REFERENCES AND NOTES

- Smith, S. B.; Finzi, L.; Bustamante, C. Direct Mechanical Measurements of the Elasticity of Single DNA Molecules by Using Magnetic Beads. *Science* **1992**, *258*, 1122–1126.
- Bustamante, C.; Marko, J. F.; Siggia, E. D.; Smith, S. Entropic Elasticity of Lambda-Phage DNA. *Science* **1994**, *265*, 1599–1600.
- Bustamante, C.; Bryant, Z.; Smith, S. B. Ten Years of Tension: Single-Molecule DNA Mechanics. *Nature* **2003**, *421*, 423–427.
- Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E. Single Molecule Force Spectroscopy on Polysaccharides by Atomic Force Microscopy. *Science* **1997**, *275*, 1295–1297.
- Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. Reversible Unfolding of Individual Titin Immunoglobulin Domains by AFM. *Science* **1997**, *276*, 1109–1112.
- Li, H.; Rief, M.; Oesterhelt, F.; Gaub, H. E. Force Spectroscopy on Single Xanthan Molecules. *Appl. Phys. A: Mater. Sci. Process.* **1999**, *68*, 407–410.
- Akaftan, O.; Tumbiolo, S.; Dubreuil, F.; Auzely-Velty, R.; Fery, A.; Papastavrou, G. Probing Multivalent Host–Guest Interactions between Modified Polymer Layers by Direct Force Measurement. *J. Phys. Chem. B* **2011**, *115*, 7726–7735.
- Li, H.; Liu, B.; Zhang, X.; Gao, C.; Shen, J.; Zou, G. Single-Molecule Force Spectroscopy on Poly(acrylic acid) by AFM. *Langmuir* **1999**, *15*, 2120–2124.
- Ludwig, M.; Rief, M.; Schmidt, L.; Li, H.; Oesterhelt, F.; Gautel, M.; Gaub, H. E. AFM: A Tool for Single-Molecule Experiments. *Appl. Phys. A: Mater. Sci. Process.* **1999**, *68*, 173–176.
- Marszalek, P. E.; Li, H. B.; Fernandez, J. M. Fingerprinting Polysaccharides with Single Molecule Atomic Force Microscopy. *Nat. Biotechnol.* **2001**, *19*, 258–262.
- Radtchenko, I. L.; Papastavrou, G.; Borkovec, M. Direct Force Measurements between Cellulose Surfaces and Colloidal Silica Particles. *Biomacromolecules* **2005**, *6*, 3057–3066.
- Rief, M.; Pascual, J.; Saraste, M.; Gaub, H. E. Single Molecule Force Spectroscopy of Spectrin Repeats: Low Unfolding Forces in Helix Bundles. *J. Mol. Biol.* **1999**, *286*, 553–561.
- Zhang, W.; Xu, Q.; Zou, S.; Li, H.; Xu, W.; Zhang, X.; Shao, Z.; Kudera, M.; Gaub, H. E. Single-Molecule Force Spectroscopy on *Bombyx mori* Silk Fibroin by Atomic Force Microscopy. *Langmuir* **2000**, *16*, 4305–4308.
- Oesterhelt, F.; Rief, M.; Gaub, H. E. Single Molecule Force Spectroscopy by AFM Indicates Helical Structure of Poly(ethylene-glycol) in Water. *New J. Phys.* **1999**, *1*, 6.1–6.11.
- Papastavrou, G.; Kirwan, L. J.; Borkovec, M. Decomposing Bridging Adhesion between Polyelectrolyte Layers into Single Molecule Contributions. *Langmuir* **2006**, *22*, 10880–10884.
- Gunari, N.; Schmidt, M.; Janshoff, A. Persistence Length of Cylindrical Brush Molecules Measured by Atomic Force Microscopy. *Macromolecules* **2006**, *39*, 2219–2224.
- Popa, I.; Zhang, B.; Maroni, P.; Schlüter, A. D.; Borkovec, M. Large Mechanical Response of Single Dendronized Polymers Induced by Ionic Strength. *Angew. Chem., Int. Ed.* **2010**, *49*, 4250–4253.
- Shi, W. Q.; Wang, Z. Q.; Cui, S. X.; Zhang, X.; Bo, Z. S. Force Spectroscopy on Dendronized Poly(*p*-Phenylene)s: Revealing the Chain Elasticity and the Interfacial Interaction. *Macromolecules* **2005**, *38*, 861–866.
- Oberhauser, A. F.; Marszalek, P. E.; Erickson, H. P.; Fernandez, J. M. The Molecular Elasticity of the Extracellular Matrix Protein Tenascin. *Nature* **1998**, *393*, 181–185.

20. Hugel, T.; Holland, N. B.; Cattani, A.; Moroder, L.; Seitz, M.; Gaub, H. E. Single-Molecule Optomechanical Cycle. *Science* **2002**, *296*, 1103–1106.
21. Yu, Y.; Zhang, Y. H.; Jiang, Z. H.; Zhang, X.; Zhang, H. M.; Wang, X. H. Full View of Single-Molecule Force Spectroscopy of Polyaniline in Oxidized, Reduced, and Doped States. *Langmuir* **2009**, *25*, 10002–10006.
22. Hugel, T.; Grosholz, M.; Clausen-Schaumann, H.; Pfau, A.; Gaub, H.; Seitz, M. Elasticity of Single Polyelectrolyte Chains and Their Desorption from Solid Supports Studied by AFM Based Single Molecule Force Spectroscopy. *Macromolecules* **2001**, *34*, 1039–1047.
23. Kirwan, L. J.; Maroni, P.; Behrens, S. H.; Papastavrou, G.; Borkovec, M. Interaction and Structure of Surfaces Coated by Poly(vinyl amines) of Different Line Charge Densities. *J. Phys. Chem. B* **2008**, *112*, 14609–14619.
24. Friedsam, C.; Gaub, H. E.; Netz, R. R. Adsorption Energies of Single Charged Polymers. *Europhys. Lett.* **2005**, *72*, 844–850.
25. Friedsam, C.; Gaub, H. E.; Netz, R. R. Probing Surfaces with Single-Polymer Atomic Force Microscope Experiments. *Biointerphases* **2006**, *1*, MR1–MR21.
26. Viani, M. B.; Schaffer, T. E.; Chand, A.; Rief, M.; Gaub, H. E.; Hansma, P. K. Small Cantilevers for Force Spectroscopy of Single Molecules. *J. Appl. Phys.* **1999**, *86*, 2258–2262.
27. Grebikova, L.; Maroni, P.; Muresan, L.; Zhang, B. Z.; Schlüter, D. A.; Borkovec, M. Interactions between Individual Charged Dendronized Polymers and Surfaces. *Macromolecules* **2013**, *46*, 3603–3610.
28. Oesterhelt, F.; Oesterhelt, D.; Pfeiffer, M.; Engel, A.; Gaub, H. E.; Müller, D. J. Unfolding Pathways of Individual Bacteriorhodopsins. *Science* **2000**, *288*, 143–146.
29. Müller, D. J.; Kessler, M.; Oesterhelt, F.; Moller, C.; Oesterhelt, D.; Gaub, H. Stability of Bacteriorhodopsin Alpha-Helices and Loops Analyzed by Single-Molecule Force Spectroscopy. *Biophys. J.* **2002**, *83*, 3578–3588.
30. Zhang, B.; Wepf, R.; Kröger, M.; Halperin, A.; Schlüter, A. D. Height and Width of Adsorbed Dendronized Polymers: Electron and Atomic Force Microscopy of Homologous Series. *Macromolecules* **2011**, *44*, 6785–6792.
31. Hugel, T.; Rief, M.; Seitz, M.; Gaub, H. E.; Netz, R. R. Highly Stretched Single Polymers: Atomic-Force-Microscope Experiments versus *Ab-Initio* Theory. *Phys. Rev. Lett.* **2005**, *94*, 048301.
32. Guo, Y.; van Beek, J.; Zhang, B.; Colussi, M.; Walde, P.; Zhang, A.; Kröger, M.; Halperin, A.; Schlüter, A. D. Tuning Polymer Thickness: Synthesis and Scaling Theory of Homologous Series of Dendronized Polymers. *J. Am. Chem. Soc.* **2009**, *131*, 11841–11854.
33. Ortiz, C.; Hadziioannou, G. Entropic Elasticity of Single Polymer Chains of Poly(methacrylic acid) Measured by Atomic Force Microscopy. *Macromolecules* **1999**, *32*, 780–787.
34. Netz, R. R. Strongly Stretched Semiflexible Extensible Polyelectrolytes and DNA. *Macromolecules* **2001**, *34*, 7522–7529.
35. Bottcher, C.; Schade, B.; Ecker, C.; Rabe, J. P.; Shu, L. J.; Schlüter, A. D. Double-helical Ultrastructure of Polycationic Dendronized Polymers Determined by Single-Particle Cryo-TEM. *Chem.—Eur. J.* **2005**, *11*, 2923–2928.
36. Zhang, A.; Rodriguez-Roper, F.; Zanuy, D.; Aleman, C.; Meijer, E. W.; Schlüter, A. D. A Rigid, Chiral Dendronized Polymer with a Thermally Stable Right-Handed Helical Conformation. *Chem.—Eur. J.* **2008**, *14*, 6924–6934.
37. Zhang, B.; Wepf, R.; Fischer, K.; Schmidt, M.; Besse, S.; Lindner, P.; King, B. T.; Sigel, R.; Schurtenberger, P.; Talmon, Y.; *et al.* The Largest Synthetic Structure with Molecular Precision: Towards a Molecular Object. *Angew. Chem., Int. Ed.* **2011**, *50*, 737–740.