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Determination of the Molar Mass of Surface-Grafted Weak Polyelectrolyte Brushes using Force Spectroscopy[⊥]

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ABSTRACT

The molar mass and dispersity of a polycation, poly[2-(dimethyl amino)ethyl methacrylate)] (PDMAEMA) grafted from a poly(methyl methacrylate) (PMMA) backbone, was measured by single-molecule force spectroscopy (SMFS) and shown to be consistent with results from gel permeation chromatography for the same comb polymer in aqueous solution. Comparison was then made between the comb polymer and PDMAEMA brushes that were grown from the substrate, as a function of the pH and ionic strength of the surrounding medium, and the limits of reliable characterization of the polymers are determined. A large discrepancy was observed between the responses of the comb and brush layer at low pH when the PDMAEMA molecules are extended from the supporting substrate. Here it is believed that the atomic force microscope (AFM) tip can penetrate the comb layer and selectively desorb side-chains of the comb. In the case of the well solvated PDMAEMA brushes at high pH, the tip preferentially selects larger chains, resulting in an over-estimate of the brush molar mass. The addition of salt also influenced the molar mass obtained by this technique. It is believed that salted brushes did not adhere well to the AFM tip, with subsequent desorption resulting in an underestimate of the molar mass. However, SMFS was shown to be capable of demonstrating the effect of salt on brush conformation, with greater swelling after the addition of a small amount of NaCl, but a significant decrease when 100 mM is added.

KEYWORDS: Force Spectroscopy, PDMAEMA, molar mass, polymer brush

INTRODUCTION

Surface-grafted polymers, known as 'polymer brushes', play a significant role in controlling the chemical, mechanical, and physical properties of a surface [1]. They are used in a wide range of applications including colloidal stabilization [2], responsive surface coatings [3], flocculation [4], and superabsorbent gels [5]. Very often they are responsive to external and/or environmental stimuli, such as electric fields [6, 7], temperature [5, 8], pH [9, 10], and salt [11, 12]. In recent years, polymer brushes have been shown to be a useful class of materials for many medical and biological applications [13, 14]. For example, custom synthetic polymers have great potential in drug delivery and molecular recognition [15], and tethering polymer chains onto surfaces can effectively reduce friction [16, 17], and control adhesion [18-20].

The use of polyelectrolyte brushes enables control of the conformational behaviour of the brush layer, when the pH or ionic strength of the medium is changed. This is usually due to changes in

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the osmotic pressure caused by the presence of counterions within the brush layer [21]. The swelling behaviour of such systems exhibits some notable characteristics depending on the pH of the solvent, concentration and type of ions in the solution, and the grafting density of the brush. In particular, much attention has been paid to weak polyelectrolyte brushes, such as those considered in the present work, because their charge density is not fixed and can be tuned by varying the ionic strength of the surrounding medium [22, 23].

In addition to a physisorption approach, chemical grafting can be achieved by two different routes: 'grafting-to', where the polymer chains are functionalized with end groups that can covalently bind to desired surfaces or interfaces, and 'grafting-from', where an initiator layer is used to grow polymer chains from a surface or interface. Forming polymer brushes via the 'grafting-to' method permits a pre-characterization of the polymers and a control of their molar mass and dispersity. However, brushes created via this method generally have a low grafting density due to entropic repulsion between the neighbouring chains. By contrast, a dense polymer brush layer can be achieved using 'grafting-from' techniques [1].

It is a considerable challenge to measure the molar mass of weak polyelectrolyte brushes prepared by the 'grafting-from' method. A simple but crude approach is to measure the height of fully stretched polymer brushes in a good solvent, and assume that this is the same as the chain length. The number averaged molar mass estimated by this method often exhibits the same order of magnitude as the values obtained by gel permeation chromatography (GPC) [24]. However, accurately determining the mass averaged and number averaged molar masses (respectively M_w and M_n), and therefore the dispersity ($D = M_w/M_n$) of the grafted chains is not trivial, given that many parameters, including grafting density, pH, and salt concentration, can affect the values obtained. In particular, due to the combination of hydrogen bonding, electrostatic interactions, and osmotic pressure from counterions, a determination of the molar mass of polyelectrolytes presents a challenge. A common method of estimating M_n and D for surface-grown polymers is to simultaneously carry out polymerization on the surface and in bulk solution under the same conditions. For this approach, one assumes that both the free and grafted polymer chains have similar molar mass and dispersity. Recently, it has been pointed out that polymers synthesized in bulk solution have a greater growth rate and a narrower molar mass distribution than those initiated from a flat substrate [25]. It has, however, been possible to characterize the molar mass from the growth of polymer from a 'free' initiator [26]. Another possibility is to remove the polymer from the surface and perform GPC on the degrafted polymer [25, 27]. This technique is restricted to the growth of polymers on small colloidal surfaces because of the need for a large surface area to permit the retrieval of sufficient polymer for the subsequent GPC analysis. Nevertheless, a different approach to effectively characterize the molar mass of surface-anchored polymers is required.

Among the few techniques that were used to characterize the molar mass of grafted polyelectrolytes, atomic force microscope-based single molecule force spectroscopy (SMFS) has been demonstrated to possess good potential [28-31]. By stretching single molecules between an atomic force microscope (AFM) tip and a supporting substrate, not only will intermolecular interactions be revealed, but also the contour length of the chain, and the conformation of the molecules on the surface or interface [10, 32-36]. The number of repeat units of each individual chain can then be estimated by dividing the calculated contour length with the length of monomer units. With the data acquisition over a large number of molecules, statistical analysis can reveal the molar mass as well its distribution. An SMFS study was carried out on grafted layers of poly(*N,N*-dimethylacrylamide), a hydrogen bonding non-electrolyte [37], where it was demonstrated that SMFS can be effective in characterizing the molar mass of grafted polymer chains of various densities. In that study it was reported that the M_n measured using SMFS agreed quite well with the GPC result, although the molar mass could be significantly underestimated when the grafting density was low. It was explained that when the distance between grafting points is greater than the radius of gyration, the polymer chains tend to form mushroom structures on the surface, increasing the probability of the AFM tip contacting points along the chain rather than contacting the chain end. In this study it was also showed that the M_w (and thus the dispersity) obtained by SMFS was considerably larger than that measured using GPC (~24%) because the AFM tip would preferentially select high molar mass chains.

The purpose of the present study is to first evaluate the effectiveness of single molecule force spectroscopy (SMFS) in characterizing the molar mass of polyelectrolyte brushes. SMFS was also used to examine the effects of salt and pH on the observed average contour length (proportional to average M_n) of films formed from a pre-characterized polymer comb [38], which also provides an effective control sample for the measurements to determine the molar mass. The comb, composed of a hydrophobic poly(methyl methacrylate) (PMMA) backbone with responsive poly[2-(dimethyl amino)ethyl methacrylate)] (PDMAEMA) side-chains, is self assembled into a brush conformation via the Langmuir-Schaefer (LS) method and deposited onto hydrophobized silicon surfaces. A homogeneous PDMAEMA brush is grafted from silicon surfaces using atom transfer radical polymerization (ATRP) [13]. For convenience, the comb refers to the polymer prepared by 'grafting-to' method, whilst brush refers to those prepared by the 'grafting-from' method, even though both methods do, in fact, produce brush surfaces. The conformational behaviour of the two materials on the supporting substrate was also revealed by SMFS. The results presented here indicate that the comb and the brush both behave differently as a function of pH and salt concentration, and successfully demonstrate that SMFS is an effective method for characterizing the molar mass of surface-anchored polymers.

EXPERIMENTAL

Materials

Silicon wafers (<100> orientation, boron doped, resistivity 0-100 Ω cm; the dopant is present at very low concentration and does not influence the brush density) were purchased from Compart Technology (Peterborough, UK). 2-(Dimethyl amino)ethyl methacrylate (DMAEMA, 98% purity, Aldrich), methyl methacrylate (MMA, 99%, Aldrich), copper(II) chloride [Cu(II)Cl_2, > 99%], 4-(dimethyl amino)pyridine (DMAP, 99%, Aldrich), triethylamine (TEA, 99%, Aldrich), anhydrous diethyl ether (Fisher), 2-Bromoisobutyryl bromide (BIBB, 98%, Aldrich) were all used as received. Copper(I) chloride (CuCl, 99%, Aldrich) was rinsed with 10% aqueous HCl, methanol, and diethyl ether in sequence, before drying in vacuum.

Preparation of PDMAEMA-coated silicon substrate

PDMAEMA chains grafted to a PMMA backbone were synthesized as described previously [38] and a PDMAEMA brush layer was formed by controlled deposition on a silicon surface, as shown in Fig. 1. The PMMA, with a molar mass of approximately 7 kDa per molecule, was used as a hydrophobic initiator backbone and then the PDMAEMA chains, with molar mass of approximately 20 kDa per chain, were grown as side-chains to form a copolymer comb. This method produced typically 2 or 3 side-chains per backbone [38]. Polymer combs were synthesized via the 'grafting-to' technique using the LS method on a silicon surface at 15 and 30 mN/m deposition pressures. The thicknesses of the resulting comb layers in this case were 1.9 nm and 3.3 nm in air as measured by ellipsometry.

As well as these comb layers, PDMAEMA brushes were grown from a silicon surface using ATRP. UV-ozone treated silicon wafers immersed overnight in a dilute anhydrous toluene solution held at -10°C of (11-(2-bromo-2-methyl)propionyloxy) undecyltrichlorosilane (the initiator for surface ATRP). The solution for surface ATRP of PDMAEMA was prepared by adding 24.1 mg CuCl, 54 µl *N*,*N*,*N*',*N*',*N*''-pentamethyldiethylenetriamine, 2.14 ml ethanol, and 2.14 ml DMAEMA to a small flask after each was sparged with nitrogen for 10 min. The treated silicon wafers were added to the solution and left at room temperature for 24 hours. Afterwards, the wafers were removed from the solution and rinsed with ethanol. The ellipsometric dry thickness of these brushes obtained from this method was 10.0 nm.

GPC characterization

The GPC setup includes two Polymer Laboratories PL gel 5 µm MIXED-C columns. The GPC eluent was HPLC grade THF containing 2.0% (v/v) TEA and 0.05% (w/v) BHT 2,6-di-*tert*-butyl-(4-methylphenol) at a flow rate of 1.0 ml/min. The temperature for the column was set at 30^0C. Calibration was carried out with ten near-uniform PMMA standards (of M_n between 2 and 300 kDa). The acquired data were analyzed using PL Cirrus GPC software (v2.0) supplied by Polymer Laboratories.

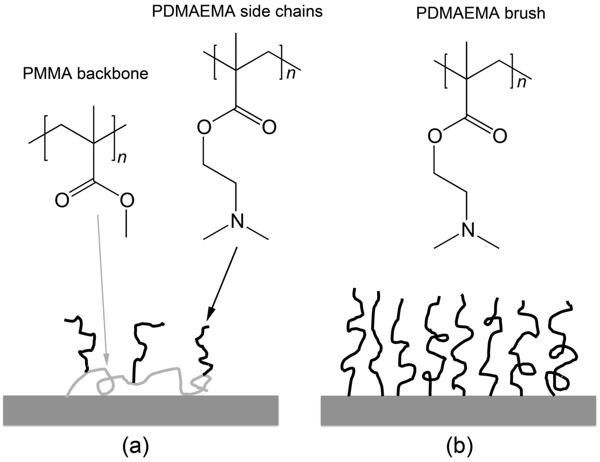

Fig. 1: Schematic diagrams showing the structure of (a) a PMMA-*graft*-PDMAEMA comb on a hydrophobized silicon surface and (b) a PDMAEMA brush grafted from a silicon surface.

Force spectroscopy

SMFS measurements were performed on films of the PDMAEMA comb polymer and brush layer grafted from the silicon substrate. A Molecular Force Probe-1D (MFP-1D) (Asylum Research, Santa Barbara, CA) was used wherein a silicon nitride AFM tip (MLCT, Veeco, Cambridge, UK) was brought into contact with a silicon substrate on which a thin film (between 2 and 10 nm dry thickness, depending on the sample used) of the polymer was grafted. Experiments were conducted in deionized water (15 MΩ resistivity, Elga PURELab option water purifier) at room temperature with a *z*-piezo velocity of 400 nm/s. Details of the MFP and the force-distance curve analysis can be found elsewhere [34, 39]. The spring constant of each cantilever was characterized using the built-in thermal calibration method [40]. During the retraction of the tip from the substrate underneath, single or multiple polymer chains were stretched, and the corresponding force as a function of distance was recorded. This elastic behaviour of the stretched single molecules can be well described by the worm-like chain model [31, 41, 42] which describes a polymer chain as consisting of N bonds with fixed length L_p joined in a linear succession,

$$F_{\text{chain}} = \frac{k_B T}{L_p}\left(\frac{D}{L_c} + \frac{1}{4\left(1 - D/L_c\right)^2} - \frac{1}{4}\right). \tag{1}$$

where k_B is the Boltzmann constant, T is the absolute temperature, D is the pulling distance, L_p is the persistence length and L_c is the contour length. Fig. 2a shows a typical pulling event fitted with the worm-like chain model.

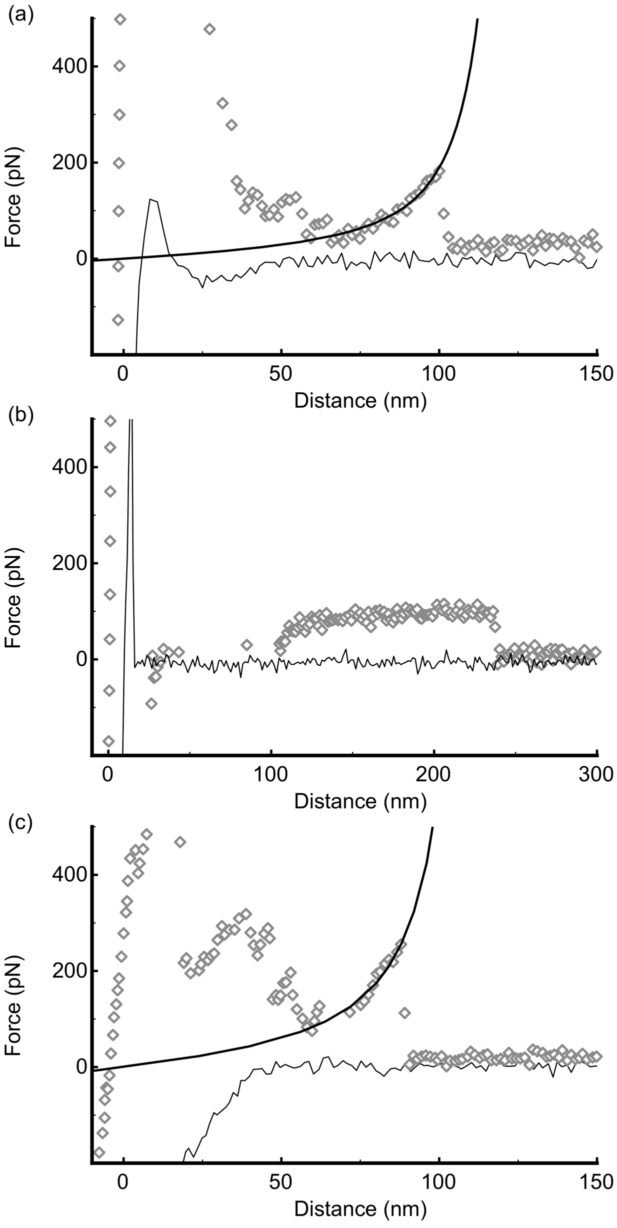

Fig. 2: Typical pulling events observed in the force curves acquired on: (a) a PDMAEMA comb layer (3.3 nm dry thickness) at pH 7 with a single peak event fitted with the worm-like chain (WLC) model (thick black solid curve), which indicates a contour length of 127 nm; (b) a PDMAEMA comb layer (3.3 nm dry thickness) at pH 7 with a plateau event; and (c) a PDMAEMA brush layer (10.0 nm dry thickness). The approach part of the curve is presented as a thin solid line in all figures.

To calculate the molar mass of the PDMAEMA comb polymer, the method developed by Al-Maawali and colleagues [29] was used in the present work. Firstly, contour lengths, L_c, were converted to molar mass using

$$M_i = \frac{M_{mon} L_c}{l}. \tag{2}$$

Here, M_{mon} and l are the molar mass and length of one 2-(dimethyl amino)ethyl methacrylate monomer unit, which are 157 g/mol and 0.28 nm (the projected C–C bond lengths in the monomer backbone) respectively. The average persistence length (a measure of chain stiffness) after WLC fitting is determined to be 0.55 nm. Because the WLC model requires that the angles at the bond junctions be fixed, but the dihedral angles are free to rotate, the persistence length cannot be used to reflect the length of monomer units. The number averaged molar mass M_{n} can then be calculated from

$$M_{\mathrm{n}} = \frac{1}{N}\sum M_{\mathrm{i}}\,. \tag{3}$$

Other than contour length, the retraction force curve also reveals the conformation of polymer chains at the solid/liquid interface. For example, a single peak shows there is only one contact point between polymer and surface, indicating that the chain is mostly immersed in the solution, with only a small *train* of monomers on the surface, whereas a plateau suggests the chain takes a *pancake* conformation. More details can be found elsewhere [10, 34, 43].

RESULTS AND DISCUSSION

Molar mass determination of the comb polymer

The molar mass of comb PDMAEMA has been determined by both GPC and SMFS methods at pH 7. As for the SMFS approach, over 100 force curves were acquired for each sample, and then processed following the methodology described in the experimental section to estimate the number averaged molar mass of PDMAEMA comb grafted on a silicon surface. Subsequently, comparison was made between the molar mass of the PDMAEMA arms of the graft copolymer acquired by SMFS and GPC, which is presented in Fig. 3 [38]. It was found that the GPC and SMFS results align remarkably well over the entire range of chain lengths. The two different sets of results show a similar distribution of M_{w}, with the peak position in the region of 51 kDa, even though GPC curve exhibits a definite tail in the higher molar mass region, which is not observed in the force measurement. While the GPC curve indicates an average M_{n} of 58.7 kDa (D = 1.17), the force spectroscopy statistics indicate an average M_{n} of 53.9 kDa (D = 1.08). This agreement is remarkably good given that it is a graft copolymer being measured. This result supports the argument that the comb chains are multi-arm, star-like graft copolymers because the contour length estimated by force spectroscopy refers to the end-to-end distance for the linear polymer chain, but the distance between the ends of two side-chains when a multi-arm polymer is being measured. This has an overall effect of making pulling events of the graft copolymer with more than two branches mimic those with only two branches, and consequently increases the frequency of two-chain-length pulling events. In the present work, the backbone of the comb polymer is quite small with respect to the side-chains and the side-chains were grafted rather sparsely, so the polymer also resembles and behaves like a star polymer. Even though using the molar mass measured by GPC to calculate the contour length of the PDMAEMA comb would be a more direct approach to verify the SMFS results, the star-like structure of PDMAEMA comb makes such comparisons difficult to interpret.

The way an AFM tip interacts with the surface-grafted comb polymer is very similar to that described in previous work [29] which showed that the polymer chains tend to slide to the side of AFM probe, with one or few contacts formed during the approach. When polymer chains are stretched away from the supporting substrate, the physisorbed chains slide on the cantilever surface until, at the end of the chain or surface, a critical number of contacts are broken which results in final rupture. It was also suggested that the rupture of contacts between polymer chains and the AFM probe, prior to the collection of monomers at the end of the chain, could shift the measured SMFS results to lower values. This would explain the discrepancy between GPC and SMFS data where a tail at higher molar mass was observed in the GPC curve.

Our results suggest that the molar mass measured by SMFS agrees well with the GPC data when only single and multiple pulling events are averaged without taking into account the plateau events (as shown in Fig. 2b) that occur when polymer chains take a pancake conformation on the solid substrate. In all cases the plateau events examined lead to a value of M_n that is between two and three times that estimated from single and multiple pulling events. This is because when the polymer chain adsorbs onto the AFM tip, there is no control over where the contact is established on the chain, and, because the whole comb is flat on the surface, invariably two or more arms are involved in the pulling event. At low pH, charging of the brush causes the longer chains of the brush to extend away from the interface and allows for enhanced tip selectivity towards higher molar mass polymers. The single and multiple peak pulling events are in best agreement with the GPC results when the brush was extended, but without excessive osmotic pressure from the counterions causing an over-estimation of the molar mass. This was achieved in solutions at intermediate pH.

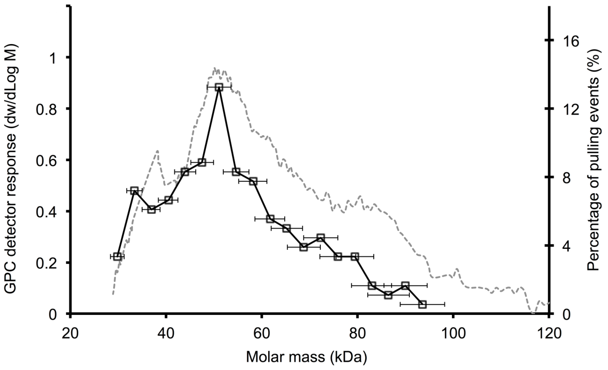

Fig. 3: Molar mass of PDMAEMA comb (3.3 nm dry thickness) at pH 7 from two different techniques, the dashed grey curve represents the molar mass distribution from the GPC method and the solid black curve represents the corresponding molar mass measured by the force spectroscopy.

pH-dependent behaviour of the swelling of the PDMAEMA comb and brush

In Fig. 4 force spectroscopy data showing the effect of the thickness and the deposition pressure during preparation of comb molecules on their interfacial behaviour (pulling distance and pulling force from a silicon surface) is presented. The two comb samples examined here possess different dry thicknesses: 1.9 and 3.3 nm respectively, which were prepared under deposition pressures of 15 and 30 mN/m respectively. Because polymer chains have to diffuse through any existing polymer film before being anchored on the substrate, low grafting densities are expected; 0.022 and 0.038 chain/nm^2 were determined for the respective deposition pressures. Fig. 4(a) shows the pulling distance distribution acquired at pH 7 for both samples. The similarity between the distributions of random pulling events suggests that there is no obvious effect of the thickness and the deposition pressure on the comb conformation as they have very similar pulling distances (~70 nm). The same conclusion can be applied to the desorption force (i.e. the force required to detach molecules away from supporting substrate), as shown in Fig. 4(b). This could well be that the grafting density of the two samples examined is within the relatively low regime of surface grafted polymer brush, therefore intermolecular interactions between PDMAEMA molecules do not have significant influence on the measured desorption force/distance.

Additionally, conformational changes of the PDMAEMA comb copolymer are presented as a function of solution pH in Fig. 4(a). At pH 7, there were more pulling events for a given data set, which suggests that the comb polymer has a greater tendency to interact with the AFM tip than in solutions of pH3. Most of the observed events were multiple pulling peaks indicating many contact points between the tip and the polymer chains. It is known that dilute PDMAEMA, with a $pK_a \approx 7$ [44], is only slightly protonated and a brush may be slightly collapsed at pH 7 [45], which makes it easier for the tip to stretch the polymer chains away from the silicon substrate because the polymer is less hydrated. The same comb was examined at pH 3, and fewer pulling events were observed; however, the pulling distance (~115 nm) is greater than that at pH 7 (~70 nm). This is because PDMAEMA chains are positively charged and highly swollen at pH 3, so the tip could have a stronger (possibly electrostatic) attraction with the molecules. At pH 10, the comb chains are completely collapsed and lie on the Si surface, and therefore only a few pulling events were observed. The limited amount of data obtained at this pH may well be due to a negatively charged (and hydrated) AFM tip being unable to interact with (possibly entangled) collapsed PDMAEMA chains. Clearly the interaction between the AFM tip and PDMAEMA brushes is dependent on the surrounding medium, and therefore the approach of determining molar mass would inevitably be affected by environmental conditions. In the case of the experiments reported here, the comparison between the GPC and SMFS data is only reliable at pH 7, which means that a degree of caution must be used when SMFS is applied to the determination of molar mass.

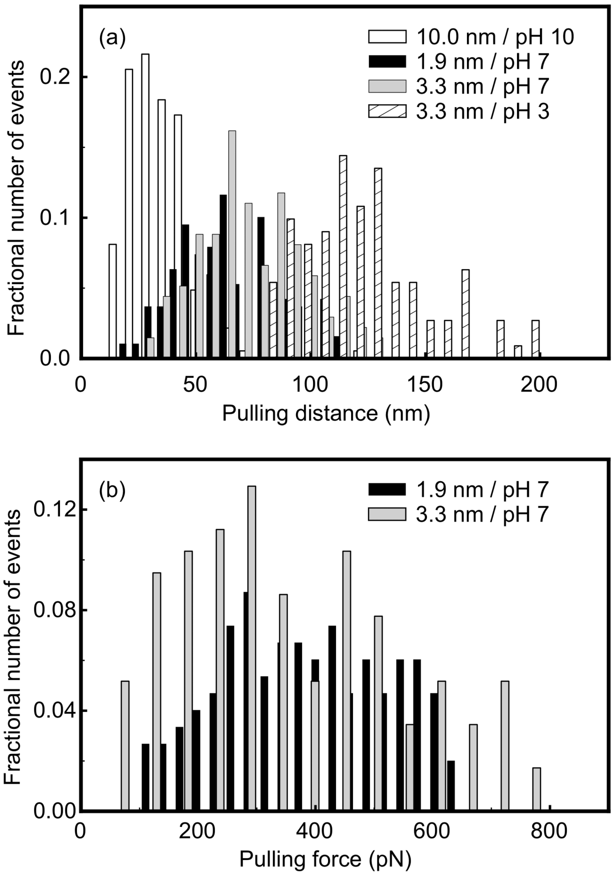

Fig. 4: (a) Distribution of pulling distances of a PDMAEMA brush grafted from a surface with a dry thickness of 10 nm at pH 10, and a PDMAEMA comb grafted to a surface with a dry thickness of 1.9 nm at pH 7, a PDMAEMA comb grafted to a surface with a dry thickness of 3.3 nm at pH 7, and a PDMAEMA comb grafted to a surface with a dry thickness of 3.3 nm at pH 3. The two histograms at pH 7 show that the thickness and the deposition pressure play no role in the swelling or collapse behaviour of the comb. (b) Distribution of desorption forces of PDMAEMA combs grafted to a silicon surface with different dry thicknesses of 3.3 nm and 1.9 nm at pH 7.

PDMAEMA brushes grafted from silicon surfaces were also examined in solutions of different pH. Fig. 4(a) shows clearly the shorter pulling distance (~30 nm) at pH 10 where the brushes were collapsed, with selectivity of the chain ends somewhat diminished compared to the stretched chains. Moreover, PDMAEMA brushes exhibited no pulling events when the solution pH was reduced from 7 to 3. The brushes are fully extended at low pH and because of the substantially greater grafting density, it is more difficult for the tip to interact with the polymer chains [46, 47].

PDMAEMA comb and brush behaviour in salt solutions

Three different forms of pulling events: single and multiple peaks, and plateaux are observed on comb polymers when exposed to 5 mM of NaCl solution at pH 3. Statistical analysis of these events is shown in Fig. 5. The pulling events indicate the way the AFM tip interacts with the polymer: single and multiple peaks suggest that there are discrete surface-chain contact points, whereas the chain was peeled off the surface when plateau events were collected. Compared to the same sample in salt-free solution at pH 3 (Fig. 4a), the single and multiple pulling distances decrease to approximately 70 nm from 115 nm with the addition of salt, whereas the dominant plateau peaks showed much longer distances (~258 nm), as shown in Fig. 5. No pulling events were observed at pH 3 when the salt concentration was further increased to 50 and 100 mM. This may be explained by considering that at pH 3 but without added salt, the silicon nitride AFM tip is slightly negatively charged, and the cationic PDMAEMA brushes are swollen and positively charged, allowing electrostatic attraction. The addition of greater amounts of salt may shield this interaction and thereby reduce the number of pulling events.

The root-mean-square thickness of polyacid close to its pK_a has been predicted theoretically to exhibit a maximum in its swelling when exposed to salt [48]. This maximum occurs with 10^{-3} volume fraction salt (or approximately 0.05 M), and was attributed to the charging effect of salt on a weak electrolyte at low salt concentrations combined with ‘salting out’ at higher concentrations, whereby counterion condensation causes the chains to become less charged. In addition, the degree of dissociation of the monomer units increased toward the brush end that extended into the solution. In a separate study, the existence of this maximum was experimentally verified for poly(methacrylic acid) brushes [21]. Although the precise grafting density of the chains was unknown, this maximum occurred at 0.002 M of $NaNO_3$.

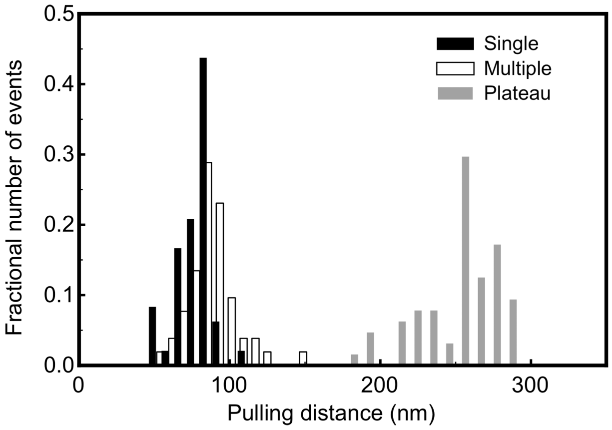

Fig. 5: Distribution of pulling distances of a PDMAEMA comb (dry thickness 3.3 nm) grafted to a Si surface at pH 3 and with an ionic strength 5 mM showing single and multiple events (70-80 nm) and plateau events (258 nm).

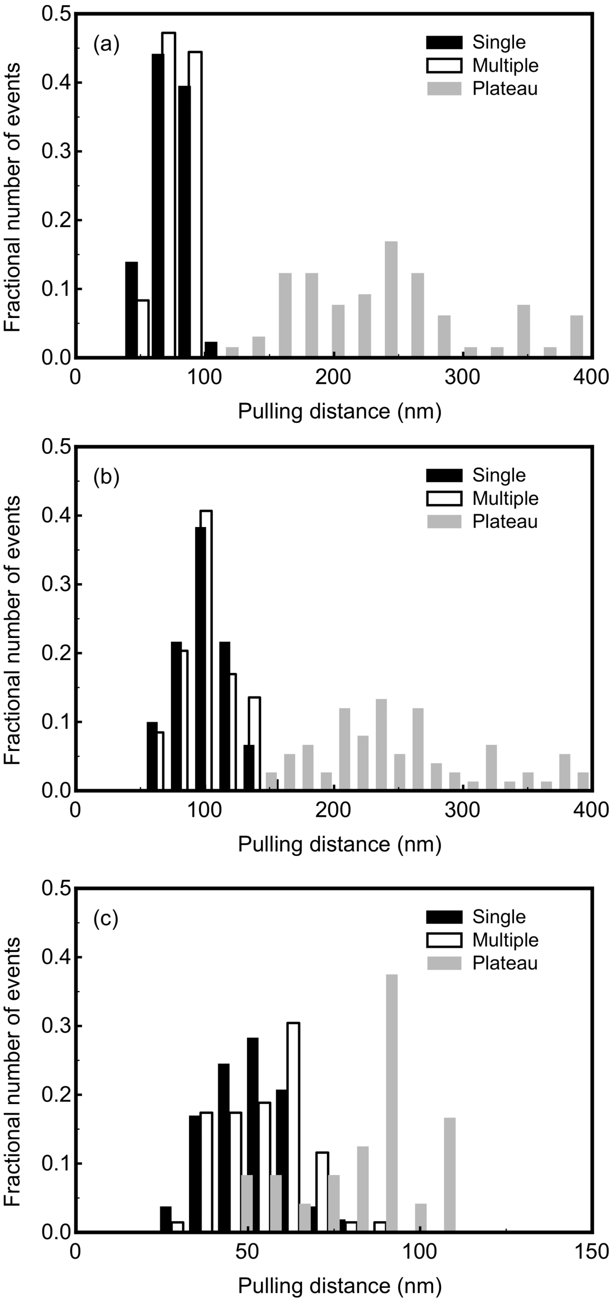

Fig. 6: Distribution of pulling distances of a PDMAEMA comb (dry thickness 3.3 nm) grafted to a Si surface at pH 7 and ionic strength: (a) 5 mM showing single, multiple events (70-80 nm), and plateau events (237 nm); (b) 50 mM showing single, multiple events (100 nm), and plateau events (260 nm); and (c) 100 mM showing single, multiple events (50-60 nm), and plateau events (90 nm).

At pH 7, pulling events were reliably and repeatedly observed at all NaCl concentrations (5, 50, and 100 mM). As shown in Fig. 6, the pulling distance increases slightly upon the addition of 5 and 50 mM of NaCl, but then decreases dramatically at 100 mM for all types of events (single, multiple, and plateau). This effect can be understood in that a sparsely grafted brush near the pK_a will swell and charge (osmotic state) upon the addition of salt, but then collapse again when reaching the salted brush regime. Also, the degree of dissociation is highest at the furthest point from the surface which could cause the tip to selectively come into contact with longer chains at higher swelling. The same salt effect was reported in other SMFS studies, e.g. in an examination of the conformation of cationic polyacrylamide on mica, it was found that the distance between

AFM tip and the last contact point on polymer chain was reduced with increased salt concentration [49].

Surface-grown PDMAEMA brushes were also examined at pH 3 upon the addition of 5, 50 and 100 mM of NaCl. Given that the brush is much more compact than the swollen comb considered above, the brush will not charge or swell readily when no salt is added. The addition of NaCl serves to swell the layer making pulling events more frequent. The pancake (plateau) conformation dominates here, although the pulling distances in this case are smaller than those for the comb at the corresponding salt concentrations (data shown in Fig. 5). The nature of the force between AFM probe and brush remains electrostatic. However, due to the increased density within the brush layer, there is much less free room available for PDMAEMA chains to rearrange their conformation. As a consequence, multiple contact points were formed on the AFM probe and the pancake conformation dominates. Fig. 7 illustrates the overall increase in the averaged pulling distances as a function of NaCl concentration (30 nm at 5 mM to 85 nm at 100 mM). This increase in the tip-sample separation indicates a general decrease in the brush layer volume as higher salt concentrations cause the brush to collapse as a result of the shielding effect.

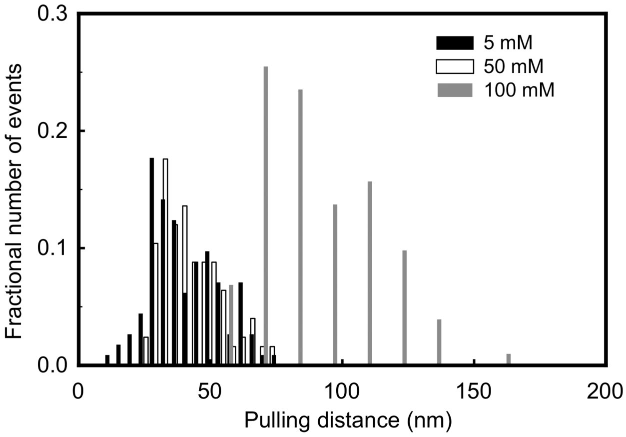

Fig. 7: Distribution of pulling distances of a PDMAEMA brushes (dry thickness 10.0 nm) grafted to a Si surface at pH 3 and ionic strengths 5, 50, and 100 mM showing the increase of pulling distance upon the addition of salt.

PDMAEMA brushes were then examined in 5, 50, and 100 mM NaCl solutions at pH 7. Fig. 8 shows that the tip-sample separation is increased compared to that at pH 3 (Fig. 7). These results indicate that the brush is entering the osmotic regime where it adopts an extended conformation. In the case of the PDMAEMA combs, it is probable that a greater salt concentration is required to reach the salted brush state (when the chains begin to collapse). Interestingly, pancake (plateau) conformations were observed more frequently in 100 mM at pH 7. This is likely to be caused by the salted brush effect that, with enough salt, causes the brush to behave as a neutral polymer. In the case of the PDMAEMA comb, which is less dense, this transition to a fully neutral brush would require the addition of more salt, whereas the highly packed brush layer experiences complete salting at 100 mM of NaCl.

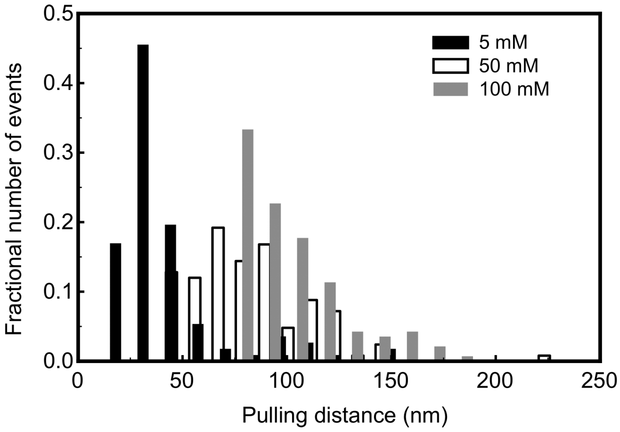

Fig. 8: Distribution of pulling distances of a PDMAEMA brushes (dry thickness 10.0 nm) grafted to a Si surface at pH 7 and ionic strengths 5, 50, and 100 mM showing the increase of pulling distance upon additional of salt.

CONCLUSION

Using a pre-characterized PDMAEMA comb polymer as a test system, the molar mass of a surface grafted weak polyelectrolyte was measured by single-molecule force spectroscopy, and was found to be in good agreement with that obtained from gel permeation chromatography. This shows that force spectroscopy has good potential in characterizing the molar mass of surface anchored polymers, at least under well controlled and understood conditions.

Statistical analysis of pulling distances and corresponding pull-off forces suggests that the deposition pressure and therefore the thickness of PDMAEMA combs have only a subtle effect on the conformation of the comb at pH 7. Furthermore, SMFS was used to evaluate the pH-responsive behaviour of surface-anchored PDMAEMA, in a comb and brush form. The tip-sample interaction in the case of the PDMAEMA comb at pH 3 is greater than that at pH 7 because the comb swells at pH 3, allowing the tip to selectively make contact with the side-chains of the comb, which also results in a greater pulling distance. On the other hand, PDMAEMA brushes showed no pulling events in this pH regime.

It was found that the addition of salt has a dramatic impact on the conformation of both PDMAEMA combs and brushes. SMFS measurements show that PDMAEMA combs swell at low salt concentrations but collapse (pancake conformation) upon an increase in salt concentration; these are respectively the osmotic and salted regimes. By contrast, PDMAEMA brushes show a continuous increase in the tip-sample separation upon the addition of salt with no loop-like conformation reported.

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