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Single Molecules and Associates of Heteroarm Star Copolymer Visualized by Atomic Force Microscopy

Anton Kiriy,*,† Ganna Gorodyska,† Sergiy Minko,*,†,‡ and Manfred Stamm†

Institut für Polymerforschung Dresden, Hohe Strasse 6, 01069 Dresden, Germany, and Department of Chemistry, Clarkson University, Potsdam, New York 13699-5810

Constantinos Tsitsilianis*

Department of Chemical Engineering, University of Patras, 26504 Patras, Greece, and Institute of Chemical Engineering and High-Temperature Chemical Processes, FORTH-ICE/HT

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ABSTRACT: Single molecule conformations and associate morphologies for polystyrene/poly(2-vinylpyridine) (PS_7−$P2VP_7$) heteroarm star copolymer, formed in controlled environment and deposited onto mica or Si wafers, have been studied by atomic force microscopy (AFM) with molecular resolution. At concentrations below 0.01 mg/mL PS_7−$P2VP_7$ exists in the molecularly dissolved state in both selective (acidic water, toluene) and common good (chloroform, tetrahydrofuran) solvents. In acid conditions PS_7−$P2VP_7$ forms either unimolecular or multimolecular micelles depending on concentration and pH. The core of the micelles is constituted of the collapsed PS arms surrounded by protonated P2VP shell. PS_7−$P2VP_7$ undergoes inverse intramolecular segregation upon addition of toluene. In this case, P2VP arms form the dense core of the micelles embedded in the swollen PS shell. The transition between those two inverse types of micelles is strongly modified by interaction with the mica substrate. The micelles deposited onto mica from acidic water are trapped via P2VP extended arms. Upon treatment of the trapped micelles with toluene the PS core is swollen and PS arms gradually adapt an extended conformation whereas P2VP trapped arms retain their extended conformation due to the strong interaction with the mica substrate. The obtained "squash" structures exhibit a unique conformation that does not exist in any solvent and could not be obtained upon a simple adsorption procedure. Surface-supported metallization of the PS_7−$P2VP_7$ on Si wafers improves AFM resolution due to the selective deposition of the Pd clusters along the P2VP chains. Thus, the number of P2VP arms of the unimers as well as the aggregation number of the multimolecular micelles and morphological details of the structures were directly analyzed.

Introduction

Star-shaped block copolymers have attracted much interest for the past decades due to their unique complex architecture that introduces novel properties different from those of their linear counterparts.[1] Heteroarm star copolymers constitute a specific type of star polymers where two different kinds of arms emanate from the same core (A_nB_n type).[2] They can be viewed as a number of diblock copolymers covalently joined together at their A−B junction points. Thus, the fixed number of diblock copolymers (defined from the synthetic procedure) will remain chemically associated in solution even in nonselective solvents at extremely low concentrations. Such a molecule undergoes diverse conformational transitions in a changing environment, representing a very promising type of a responsive material for the fabrication of smart polymer films, micelles,[3] and drug delivery systems.

Most of recent investigations on morphology of structures formed by molecules with a complex architecture were done with scattering technique when all details of morphology cannot be easily extracted in the case of the complex structures. Atomic force microscopy (AFM)[4] has been proved as a powerful tool for the visualization of biological or synthetic giant and rigid[5] or even "normal" flexible[6,7] single polymer molecules. AFM with molecular resolution allows to follow conformational transformations of polymeric molecules, and even in some cases it allows to perform a quantitative analysis of chain statistics.[5] Recently, we have reported the visualization of polysterene/poly(2-vinylpyridine) heteroarm star copolymers (PS_7−$P2VP_7$, where 7 is the average number of arms, Table 1) and single molecules deposited on the mica substrate from different environments.[7e] The present paper is the extended report of the study and contains a new material on the conformational transitions of the deposited molecules and on morphology of the associates.

Table 1. Characteristic of the PS_7−$P2VP_7$ Sample

$M_w(PS_{arm})$/no. of arms	by SEC/calcd	20000/6.9
$M_w(P2VP_{arm})$/no. of arms	by LS/calcd	56500/6.9
W_{P2VP}, fraction of P2VP	by NMR	66%
$M_w(PS_7−P2VP_7)$	by LS	544 000

We explore the AFM experiments with single molecules deposited from very diluted solution onto a solid substrate. The molecules are scanned in the tapping mode after a rapid evaporation of solvent. A correct interpretation of the obtained results requires to take care on possible changes in conformations introduced by the AFM experiment itself due to the interaction with the tip or due to the deposition procedure. The first can be relatively easy controlled, while the second makes a real problem for the application of AFM in single polymer molecule study. We address the question how large are changes in the conformation for the deposited molecule as compared to the conformation in solution? These changes depend strongly on history of the sample and on the specific behavior of the particular system so

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† Institut für Polymerforschung Dresden.

‡ Clarkson University.

* Corresponding authors: E-mail: kiriy@ipfdd.de; sminko@clarkson.edu; ct@chemeng.upatras.gr.

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Table 2. Average Dimensions of the PS_7-P2VP_7 (on the Basis of at Least 100 Structures Taken from 5 to 10 AFM Images for Each Condition)

	shell		core	
conditions	diameter, nm	height, nm	diameter, nm	height,[a] nm
$CHCl_3$[b]	46 ± 8	1 ± 0.2	18 ± 4	2.6 ± 0.2
THF[b]	36 ± 10	2.4 ± 0.5		
pH 2[b]	126 ± 35	0.3 ± 0.1	17 ± 7	2.9 ± 0.3
Pd-metallized unimolecular micelles	122 ± 30	1 ± 0.2	9 ± 2	7.2 ± 2
pH 2, Na_3PO_4, 0.2 mg/mL[b]	99 ± 15	1 ± 0.2	15 ± 5	5.6 ± 0.5
pH 3.5 (unimers, 81%/micelles, 19%)	119 ± 30	0.3 ± 0.1	10 ± 2/20 ± 4	4.9 ± 0.5/16 ± 3
pH 4.2 (micelles)[b]	137 ± 35	0.3 ± 0.1	22 ± 5	17 ± 1
pH 1, 0.3 g/L of PS_7-P2VP_7 (micelles)			34 ± 5	15.9 ± 3
pH 1, 0.1 g/L of PS_7-P2VP_7 (micelles)			30 ± 5	12.5 ± 3
pH 1, 0.03 g/L of PS_7-P2VP_7 (micelles)	195 ± 40	0.4 ± 0.1	25 ± 5	12.1 ± 3
Pd-metallized multimolecular micelles	151 ± 50	1 ± 0.2	40 ± 10	25.4 ± 5
toluene, 3 min[b]	55 ± 5	0.6 ± 0.2	44 ± 5	2.9 ± 0.3
toluene, 30 min[b]	80 ± 10	0.5 ± 0.2	36 ± 10	2.4 ± 0.3
pH 2, adsorption, then toluene, 30 min	129 ± 40	0.4 ± 0.1	41 ± 10	2.3 ± 0.5
pH 2, adsorption, then toluene, 5 h	140 ± 50	0.8 ± 0.5		

[a] Measured from basic line on the mica surface. [b] 0.001 g/L of PS_7-P2VP_7.

that there is no general answer, and the interpretation of the results should be referred to each particular system. Study of the molecules with a complicated architecture helps very much in solution of the problem as we show in this report. We would like to reconstruct the solution conformation visualizing the conformation of the molecules deposited on a solid substrate. Thus, we focus here on the trapped conformation of the molecules which is as far as possible from the equilibrium conformation of the adsorbed molecules.

As it has been reported earlier, the PS_7-P2VP_7 copolymers self-assemble in the bulk[8] due to incompatibility of the different arms as well as they form micelles in selective solvents.[9] In the latter case it was found that the critical micelle concentration (cmc) of the copolymer is much higher than that observed for the linear block copolymers, showing that the single star molecules survive (no association) at relatively low concentrations.[9] An interesting property of this copolymer is that the P2VP arms can be protonated in acidic media, and thus, they can be transformed to a weak cationic polyelectrolyte. Therefore, the copolymer is transformed to an amphiphilic state upon protonation. Since the hydrophilic part is a weak polyelectrolyte, it responds to external stimuli such as pH and ionic strength.

In this work we describe a variety of structures formed by the copolymer due to the interplay of the van der Waals and electrostatic interactions in different environment and with the mica substrate.

Experimental Section

Materials. The PS_7-P2VP_7 heteroarm star copolymer was prepared via a three-step sequential "living" anionic polymerization procedure using divinylbenzene linkage (DVB).[10] In the first step the polystyrene arms were prepared using *sec*-butyllithium as the initiator at −40 °C in THF. After the consumption of the styrene monomer and sampling out, a small amount of divinylbenzene was added to the reaction medium. Star-shaped polystyrene (PS_n) was thus formed, part of which was deactivated and sampled out for the purpose of characterization. The remaining "living" star polymer was used to initiate the polymerization of a chosen amount of 2-vinylpyridine that was added to the reaction medium at −75 °C. After complete polymerization of 2-vinylpyridine the reaction mixture was deactivated with degassed methanol, and the final product was recovered by precipitation in cold heptane, dried, redissolved in benzene, and freeze-dried. The molecular characteristics of the copolymer are given in Table 1.

Samples Preparation. Solution of PS_7-P2VP_7 in THF (0.01 g/L) was diluted by slow addition of the 10-fold excess of either acidic (pH 1, 2, 3.5, or 4.2, HCl, Aldrich), water (Millipore water, 18 MΩ·m cm), toluene, or chloroform. For experiments with salt 1 mL of PS_7-P2VP_7 solution in THF (0.01 g/L) was diluted by slow addition of 8 mL of acidic water (pH 2) and then 1 mL of 10 mM Na_3PO_4 (Aldrich). After stirring (in the most cases for 2 h) we set a drop of the solution on the surface of freshly cleaved mica (isoelectric point at pH 3.0) for 1 min and afterward removed the rest of the drop with weak centrifugal force. The dry samples were investigated with AFM. For reconformation-on-the-surface experiments PS_7-P2VP_7 have been, first, adsorbed from the acid water solution and dried. In the second step, we set a drop of toluene on this sample and covered it with a Petri glass to prevent the solvent evaporation. Afterward, samples taken in different periods of time were dried and investigated with AFM.

Metallization on Si Wafers. Highly polished Si wafers (obtained from Wacker-Chemitronics) were first cleaned in a ultrasonic bath three times for 5 min with dichloromethane (DCM) and placed in the cleaning solution prepared from NH_4OH and H_2O_2 at 60 °C for 1 h. (Note: the NH_4OH:H_2O_2 solution reacts violently with organic compounds. Caution should be used when handling this solution.) Samples were finally exposed to 50% sulfuric acid for 15 min and then rinsed several times with Millipore water (18 MQ cm). Na_2PdCl_4, dimethylamine borane (DMB), and DCM were used as received from Aldrich. A solution of PS_7-P2VP_7 in THF (0.01 g/L) was diluted by slow addition of the 10-fold excess of acidic water (pH 2, HCl). After stirring for 120 min we set a drop of the solution on the surface of the cleaned Si wafer for 1 min and afterward removed the rest of the drop with a centrifugal force. Then, in the second step, the Si wafer was rinsed several times with water and placed into the Na_2PdCl_4 solution (pH 2, HCl) for 1 min for the formation of the $P2VPH^+\cdots(PdCl_4)^{2-}$ composite. In the third step the Si wafer was thoroughly rinsed with water and placed for 15 s into reduction solution which contained 1.0 mg/mL of DMB. Finally, the Si wafer was cleaned with water and dried with an argon flux.

AFM Measurements. A multimode AFM instrument (Digital Instruments, Santa Barbara, CA) was operating in the tapping mode. Silicon tips with radius of 10−20 nm, spring constant of 30 N/m, and resonance frequency of 250−300 kHz were used after calibration with gold nanoparticles (of diameter 5 nm) to evaluate the tip radius. The dimensions of structures obtained from AFM images were corrected (decreased) by the tip radius.[11] To evaluate the volume (V) of the visualized structures for simplicity we used the following formula for the cone shape: $V_{\mathrm{cone}} = {}^{1}/_{3}H\pi(D/2)^2$, where H and D are the height and diameter of structures observed with AFM and presented in Table 2. The values were compared with the calculated volume based on molecular composition of the

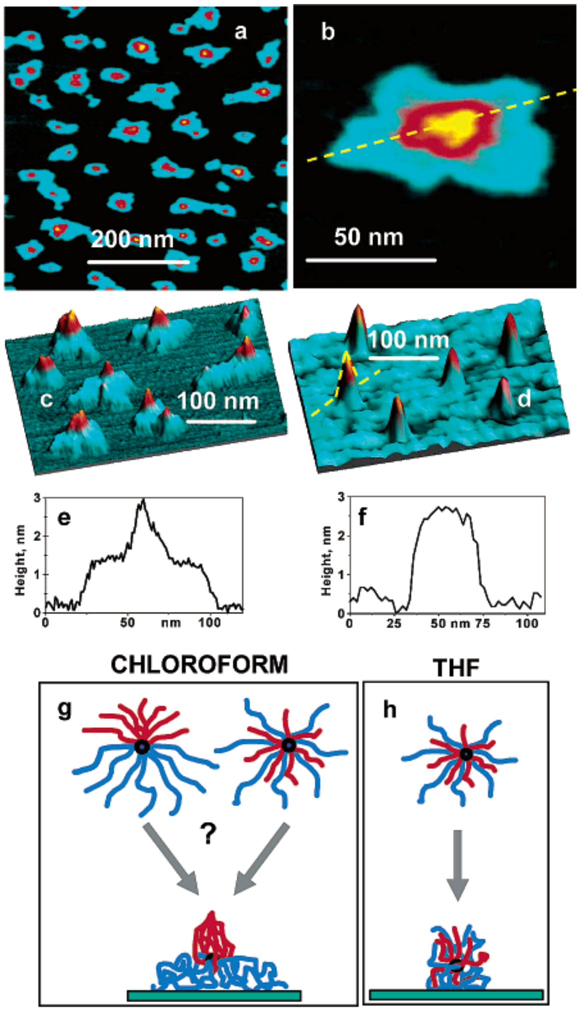

Figure 1. Representative AFM topographic images (a−c) and cross sections (e, f) of the PS_7-P2VP_7 adsorbed from chloroform. 3D-AFM image (d) and cross section (f) of unimers adsorbed on mica from THF. Schematic representation of the solution conformations and conformations in adsorbed state of the PS_7-P2VP_7 in chloroform (g) and THF (h), respectively (red color indicates PS arms).

copolymer: $V_{PS,calc} = 7N_{PS}\,M_S\,\rho_{PS}/N_A = 7 \times 190 \times 105 \times 1.05/6 \times 10^{23} = 245\ \mathrm{nm}^3$, where N_{PS} is the degree of polymerization of PS arms, M_S is the molecular mass of styrene unit, ρ_{PS} is the density of PS, and N_A is Avogadro's number. A similar calculation for P2VP arms give $V_{P2VP,calc} = 698\ \mathrm{nm}^3$, and the total volume per molecule $V_{PS_7-P2VP_7,calc} = 943\ \mathrm{nm}^3$.

Result and Discussion

Deposition from Common Good Solvents. Chloroform and tetrahydrofuran (THF) are good solvents for both P2VP and PS components, and PS_7-P2VP_7 molecules are expected to be molecularly dissolved. However, the molecules deposited on the mica surface from very dilute solutions show different conformations for those two solvents. The molecules deposited from chloroform were observed to form hatlike structures, consisting of a very flat bottom layer about 1 nm in height and 48 nm in diameter and a semispherical top layer near 2.6 ± 0.2 nm in height (H) and 18 ± 4 nm in diameter (D) (Figure 1a−c,e and Table 2). In this case and below for simplicity in the discussion we represent all structures formed by the copolymer deposited on the mica surface as an apparent core−shell structure, where a compact fragment formed by collapsed arms we take as a core, while extended arms we take as a shell.

Table 3. Solubility Parameters

polymer/solvent (subscripts)	solubility parameter $(\mathrm{cal/cm^3})^{1/2}$/ difference of solubility parameters
δ_{PS}	9.1
δ_{P2VP}	9.9
δ_{CHCl_3}	9.33
δ_{THF}	9.51
$(\delta_{PS} - \delta_{THF})^2$	0.16
$(\delta_{P2VP} - \delta_{THF})^2$	0.15
$(\delta_{PS} - \delta_{CHCl_3})^{1/2}$	0.32
$(\delta_{P2VP} - \delta_{CHCl_3})^{1/2}$	0.05

However, when PS_7-P2VP_7 molecules are deposited on the surface from THF solution, near-uniform spherical particles with $D = 36 \pm 10$ nm and $H = 2.4 \pm 0.5$ nm (Figure 1d,f and Table 2) are formed. We may speculate that this difference in morphology of the structures from THF and chloroform might be attributed to different conformations in solution. It seems that in chloroform an intramolecular segregation occurs, leading to "Janus"-like star copolymers (Figure 1g), whereas in THF the different arms are distributed randomly around the star core (Figure 1h). The phenomenon of block copolymer segregation in common good solvents has been observed indirectly by viscometric measurements in diblock and also in star copolymer dilute solutions.[12] Also, we mention here some analogy of the heteroarm star block/copolymer to mixed polymer brushes on a flat substrate. The mixed brushes demonstrate the rich phase behavior. Change of solvent selectivity results in switching between different phases, e.g., from disordered state to laterally segregated or perpendicular (to the substrate) segregated phases.[13]

To support that assumption, we present the solubility parameters for PS and solvents (Table 3) found in the literature[14] and the solubility parameter for P2VP calculated from the interaction parameter $\chi_{PS-P2VP} = 0.1$[15] using the equation $\chi_{PS-P2VP} = [V_r(\delta_{PS} - \delta_{P2VP})^2]/RT$, where V_r is the molar volume of P2VP (98 $\mathrm{cm^3/mol}$); δ_{PS} and δ_{P2VP} are the solubility parameters for PS and P2VP, respectively. The differences of the solubility parameters $(\delta_{polymer} - \delta_{solvent})^2$ presented in Table 3 demonstrate that chloroform is a more selective solvent (for P2VP) than THF. Hence, chloroform may promote phase segregation in the star copolymer schematically shown in Figure 1g.

Once a copolymer molecule approaches the mica surface, the P2VP arms are oriented toward the substrate due to their high affinity. Such a mechanism of adsorption results in the observed structures after rapid evaporation of chloroform. Thus, the major P2VP constituent (66 wt %) is located on the surface forming the flat bottom layer while the minor PS constituent is located on the top forming the semispherical bump (Figure 1g). On the other hand, in THF segregation of the arms cannot be resolved in the AFM experiments, indicating the formation of phases of a smaller characteristic size (Figure 1h).

To prove that the observed structures represent single molecules, we evaluated an experimental average volume of the structure and compared with the values calculated from molecular mass of the polymers. The calculated values 698, 245, and 943 nm^3 are for P2VP arms, PS arms, and total volume of the copolymer molecule, respectively. We assume that PS arms are not trapped on the surface and collapse to form structures with the bulk density. Thus, we obtained the experimental volume for the PS core, if the copolymer is

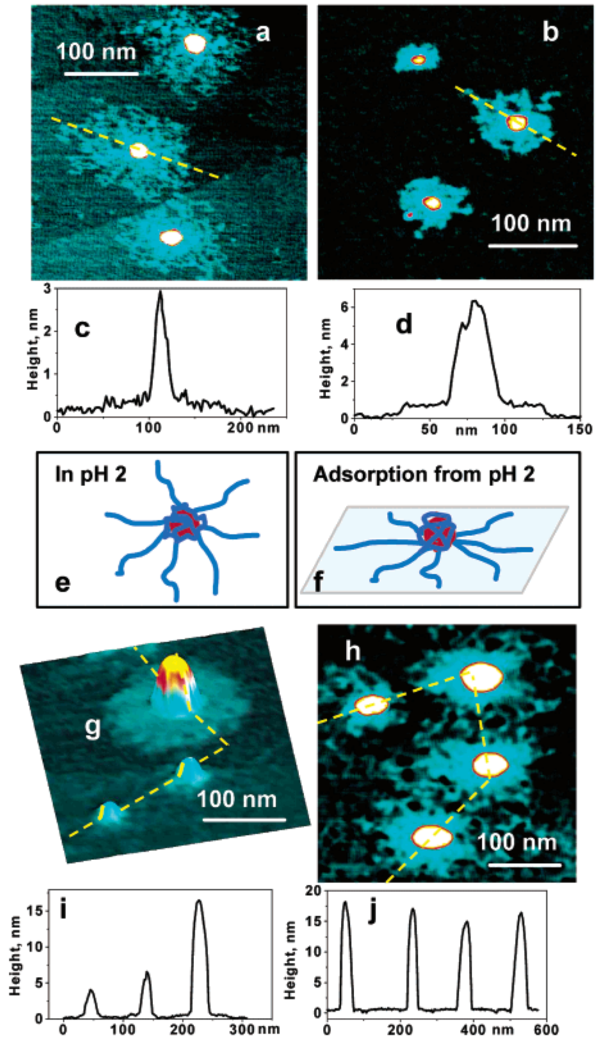

Figure 2. AFM topographic images (a, b, g, h) and cross sections (c, d, i, j) of PS_7-P2VP_7 adsorbed from acid water (pH 2, HCl): in salt-free condition (a, c) and in the presence of 1 mM of Na_3PO_4 (b, d). Schematic representation of the unimolecular micelle formed in acid water: in solution (e); in adsorbed state (f). 3D image (g) and cross section (i) of micelles with different aggregation number formed at pH 3.5. AFM images (h) and cross section (j) of multimolecular micelles adsorbed from acid water (pH 4.2, HCl).

deposited from chloroform, to be 220 nm^3, which is in good agreement with the calculated value.

Aqueous Solutions: Effect of pH and Ionic Strength and Polymer Concentration. It was previously shown that at a relatively high concentration (0.3 g/L) PS_7-P2VP_7 undergoes intermolecular micellization in acid water (pH 1−2) with the aggregation number equal to 8.[9c] In contrast, at extremely low concentrations (0.005 g/L of PS_7-P2VP_7) micellization occurs as an intramolecular process. In these conditions the PS_7-P2VP_7 star copolymer survives in the nonassociated state and forms stable unimolecular micelles. Figure 2 shows representative AFM images of mica-deposited uniform star-shaped core−shell structures formed upon dilution of PS_7-P2VP_7/THF solution with acid water (pH 2). Such a morphology reflects very pronounced intrasegregation of the star copolymer in acid water.[16] PS chains collapse due to hydrophobic interactions and form a compact core whereas protonated P2VP arms adopt an extended conformation due to the Coulomb repulsion and form a shell (Figure 2e). We may conclude that the AFM image represents an "off print" of the solution conformation when the molecules were adsorbed and trapped by the substrate. After the rapid evaporation of water the structure was formed due to the collapse in the *Z*-direction (Figure 2f). The experimental value of the collapsed PS core volume is about 219 nm^3, which again corresponds to the calculated value (245 nm^3).

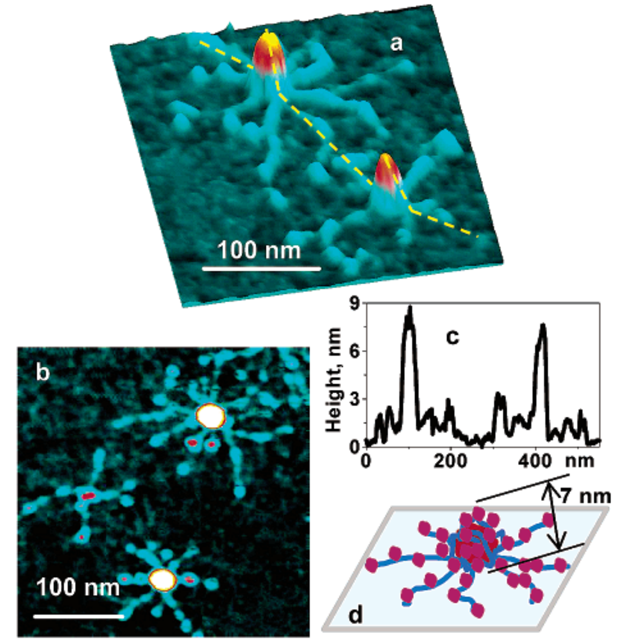

Figure 3. 3D (a) and 2D (b) AFM images, cross section (c), and schematic representation (d) of Pd-metallized unimers.

To improve AFM resolution of P2VP arms, we employed a recently developed approach to decorate polyelectrolyte chains with metal clusters. This method consists of the ion-exchange reaction between protonated $(P2VPH^+)Cl^-$ and Na_2PdCl_4 and the following reduction of Pd with dimethylamine borane.[7b,c,e] After metallization PS_7-P2VP_7 deposited on the Si wafer from acidic aqueous solution with fully extended P2VP arms appears in very good resolved star-shaped conformations (Figure 3). The P2VP arms decorated with Pd clusters are clearly observed in the AFM images and can be counted (Table 4).

The AFM images for the first time visualize the second generation of the P2VP arms, which were growing up from the active sites located on the DVB core of the PS star polymer precursor. Although the first generation of the PS arms was characterized (number of arms) by light scattering from the molecular weights of the star copolymer and the arms (sampling out before adding DVB), the characterization of the P2VP arms was performed only due to the visualization. The average number of P2VP arms was counted directly from the AFM images to be 7 ± 1.26 (Figure 4), which was in excellent agreement with the number of PS arms found by light scattering. This result gives unambiguous evidence that the number of the chemically different arms is equal. At the same time, that is very exciting example how AFM visualization can be used for analysis of polymer architecture.

Metallization of the adsorbed PS_7-P2VP_7 molecules allowed us to get better insight into the structure of the unimolecular micelles. Specifically, we found the considerable increase of the height of the cores occurred

Table 4. Dimensions of Pd$\cdots$PS$_7$–P2VP$_7$ Nanocomposites (Averaged from 52 Structures in Six AFM Images)

length (L), height (H), and no. (N) of P2VP arms			diameter (D) and height (H) of PS core		diameter (D), height (H), and no. (N) of Pd clusters per arm		
L, nm	H, nm	N	D, nm	H, nm	D, nm	H, nm	N
61 ± 15	1 ± 0.2	7	9 ± 3	7.2 ± 2	3 ± 1	2.2 ± 1	3 ± 1

upon metallization (from $H = 2.9$ nm before metallization to $H = 7-12$ nm after, Table 2). Ion-exchange reaction of palladium tetrachloride dianion serves a selective and efficient probe on polycations, and thus, such an observation clearly indicates that pyridine units are localized in the shell and in the core of the micelles (Figure 3d).

Na_3PO_4 is a strong condensation agent for PE. A coil–globule transition for a cationic PE was documented at 10–15 mM of Na_3PO_4.[7d] In this study, a full precipitation of PS$_7$–P2VP$_7$ from acid water (pH 2) solution at even lower concentration of Na_3PO_4 (5 mM) was observed. However, at 1 mM Na_3PO_4, PS$_7$–P2VP$_7$ molecules survive in the nonassociated state with a contracted shell ($D = 99 \pm 15$ nm instead of $D = 126 \pm 35$ nm in salt-free conditions; Table 2, Figure 2b). On the other hand, the size of the hydrophobic core increases ($H = 5.6 \pm 0.5$ nm instead of $H = 2.9 \pm 0.3$ nm in salt-free conditions), indicating that uncharged P2VP arms condense on the core. This confirms the suggestions deduced indirectly from light scattering and fluorescence measurements reported previously.[9c] This experiment gives additional evidence that the deposited structures analyzed with AFM precisely reflect the prehistory in the solution.

Unimolecular micelles were found to be stable in dilute solutions at pH 2 for at least several days. At pH 3.5 about 20% of PS$_7$–P2VP$_7$ molecules undergo association, leading to multimolecular micelles, whereas most of molecules remain unassociated (unimers) with slightly compressed shells (Table 2). Figure 2g,i shows clearly the coexistence of micelles and unimers with significant differences on their size. At pH 4.2, the association phenomena lead to multimolecular micelles (Figure 2h,j). The size of the PS cores ($H = 17 \pm 1$; $D = 22 \pm 5$) has been increased significantly as compared to unimolecular micelles. The association is also reflected in an increase of the diameter of the shell ($D = 137 \pm 35$) if more P2VP chains are accumulated in the corona, resulting in larger stretching due to the excluded-volume effect. The formation of micelles at pH 4.2 (Figure 2h,j) may be attributed to the fact that a considerable part of P2VP exists in nonprotonated state, altering the hydrophobic–hydrophilic balance of the copolymer and therefore destabilizing the unimers inducing association.

At concentrations higher than 0.01 g/L in acid water (pH 1) PS$_7$–P2VP$_7$ forms aggregates. As expected, the increase of the polymer concentration leads to the increase of the aggregation. Although most of the structures formed at concentration 0.03 g/L are unimolecular micelles, a 10 times concentrated solution demonstrates a comparable amount of multimolecular micelles and unimers (Figure 5). Metallization by Pd improves the AFM contrast and allows to estimate the aggregation number by the direct counting of the metallized arms (Figure 6b). As in the case of unimers, metallization of the multimolecular micelles leads to the increase of the height of the core from 10 to 20 nm before metallization to 25–35 nm after metallization.

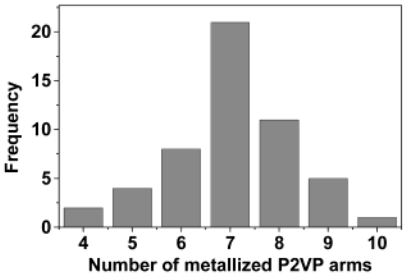

Figure 4. Distribution of number of metallized P2VP arms (taken from 52 structures in six AFM images).

Solutions in a Selective Organic Solvent. It is well documented that the critical micelle concentration (cmc) of similarly designed PS$_6$–P2VP$_6$ copolymers in toluene is near 0.7 mg/mL and that at concentrations below cmc PS$_6$–P2VP$_6$ forms inverse unimolecular micelles.[9a] AFM experiments confirm that for PS$_7$–P2VP$_7$. Immediately after addition of toluene (good solvent for PS arms and bad solvent for P2VP arms) into the PS$_7$–P2VP$_7$/THF solution, intramolecular segregation occurs rather than aggregation. Unimers ini-

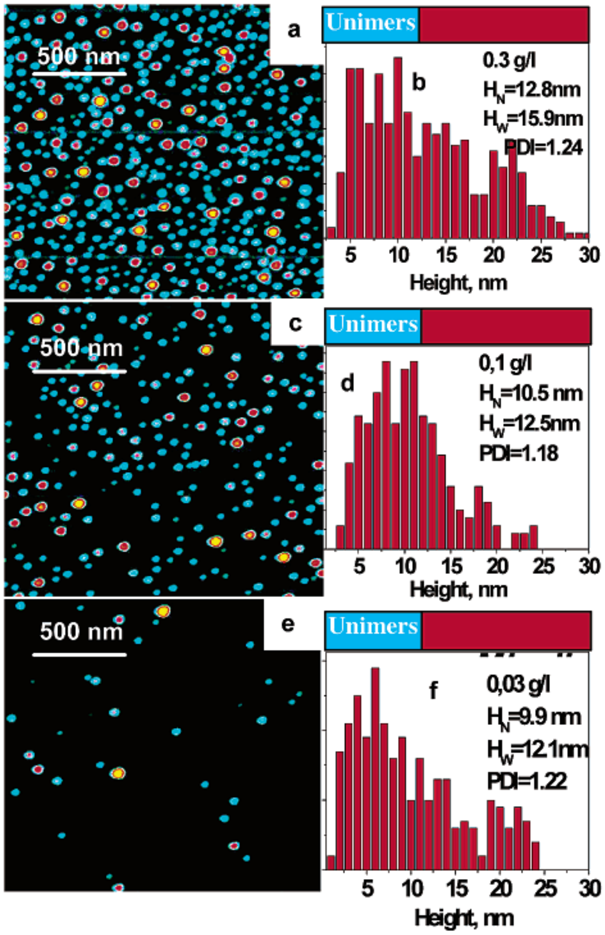

Figure 5. AFM images and height distribution diagrams of micelles obtained in acid water (HCl, pH 1) at different concentration of PS$_7$–P2VP$_7$: 0.3 g/L (a, b); 0.1 g/L (c, d); 0.03 g/L (e, f). All structures with the height larger as 10 nm we consider as associates.

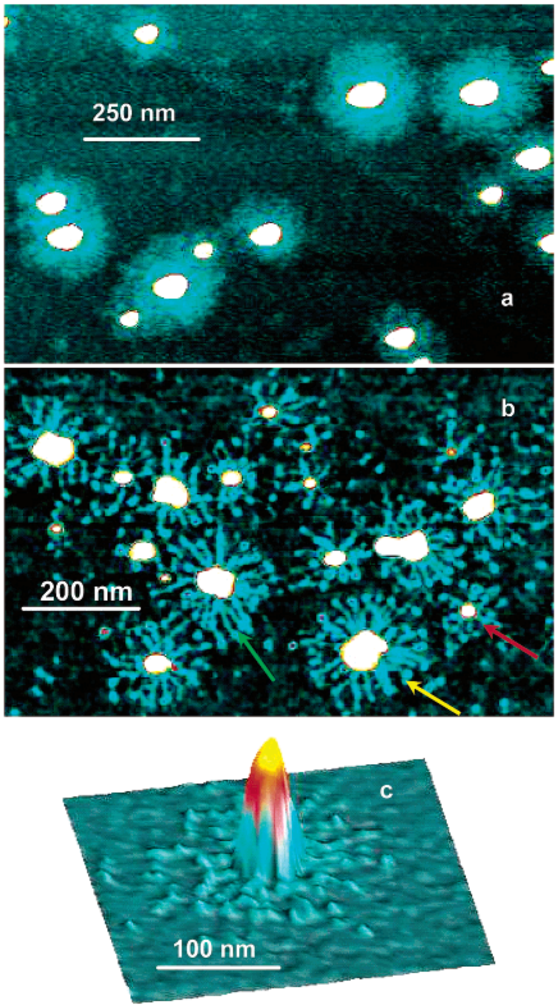

Figure 6. Coexistence of uni- and multimolecular micelles at concentration of PS_7-P2VP_7 0.03 g/L (HCl, pH 1) before (a) and after metallization with Pd (b, c). Metallization improves AFM contrast, and thus the aggregation number can be directly estimated. For example, the structure marked by the yellow arrow corresponds to a tetramer with about 28 P2VP arms, the green arrow corresponds to a trimer with about 21 P2VP arms, and the red arrow shows a unimer with seven P2VP arms.

tially formed upon addition of toluene are poorly segregated particles consisting of swollen cores ($D = 44 \pm 5$ nm) and small shells ($D = 55 \pm 5$ nm, Table 2; Figure 7a,c). A full reconformation of the unimers occurs within 1 h. Starlike structures with well-definite PS arms (about 30 nm in length) in the shell and P2VP arms collapsed in the core were observed (Figure 7b,d). The core diameter is $D = 36 \pm 10$ nm, about twice of the PS core that is observed for the unimolecular micelles in water. The latter is consistent with the composition of the copolymer ($W_{P2PV}/W_{PS} \approx 2$). Moreover, the P2VP core volume calculated from AFM data is $V_{AFM} = 813$ nm^3, which is slightly higher than the volume of P2VP blocks calculated from the degree of polymerization ($V_{P2VP,calc} = 698$ nm^3). That provides the evidence for the formation of the inverse unimolecular micelles upon addition of toluene.

Reconformation on the Surface. Recently, we found that protonated single P2VP chains can be frozen in extended conformation upon adsorption onto the mica surface.[4a,d] Such adsorbed polycation molecules retain their initial conformation for a long period of time even after the exposure to solutions of a high ionic strength. This effect has been attributed to strong attractive interactions between P2VP chains and mica. On the other hand, we have found that adsorbed hydrophobic polymers interact weakly with the mica surface and alter their conformation responding to environmental changes. Exploring this different behavior of P2VP and PS chains on the mica surface, we produced a unique conformation of the block copolymer which does not exist in any solvent and could not be obtained upon an adsorption procedure. First, PS_7-P2VP_7 molecules were adsorbed onto mica from acid water in the star-shaped conformation with collapsed PS blocks and extended P2VP chains (Figure 2a). Thereafter, the mica substrate with the deposited copolymer was placed into toluene. The absorbed PS_7-P2VP_7 molecules gradually changed their conformation (Figure 8a−f) via swelling and extension of the PS arms. The P2VP arms retained their conformation unchanged. Finally, the PS_7-P2VP_7 molecules formed very flat “squash” structures. Multimolecular micelles deposited onto the mica surface undergo similar reconformation (Figure 8g,h). During the transformation the height of the core decreases (from 10 to 20 nm to 2−3 nm) whereas the diameter of the core increases in several times (up to 100 nm).

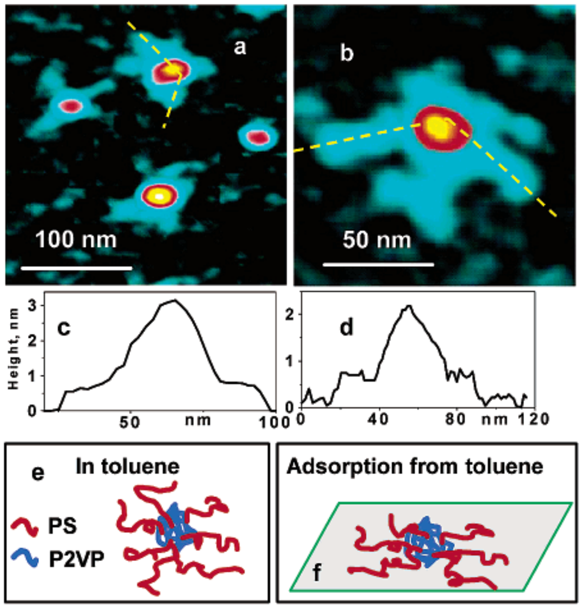

Figure 7. AFM images (a, b) and cross section (c, d) of reverse unimolecular micelles formed upon addition of toluene in THF solution: after 3 min (a, c); after 30 min (b, d). Schematic representation of the reverse unimolecular micelle formed in toluene: in solution (e); in adsorbed state (f).

Similar experiments we performed for the metallized structures. Initially, we have deposited the unimers and multimolecular micelles on the Si wafer. Afterward, we metallized the structures and exposed them to toluene. In contrast, the *metallized structures* (unimers and multimolecular micelles) *only partially decreased* their height upon exposure to toluene during several hours (from 7 to 12 nm to 4−6 nm for unimers and from 25 to 35 nm to 15−30 nm for multimolecular micelles; Table 2). Obviously, such a decrease is due to the reconformation of PS blocks from the collapsed state into the more extended state. The remaining material located in the center of the micelles, most likely, corresponds to the Pd clusters in the core of the micelles.

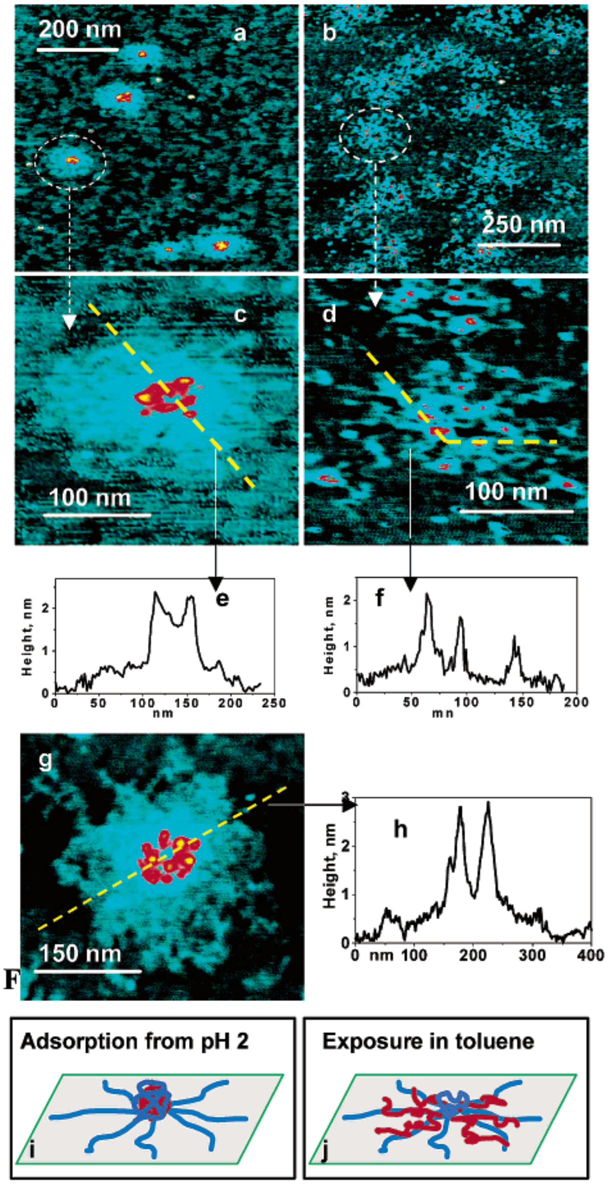

Figure 8. AFM images (a–d) and cross sections (e, f) of the PS_7–$P2VP_7$ unimers (a–f) and multimolecular micelles (g, h) adsorbed from acid water (pH 2, HCl) onto the mica and then placed in toluene for 30 min (a, c, e) and 5 h (b, d–h). Schematic representation of the unimolecular micelle adsorbed from acid water (i) and formed upon exposure to toluene (j).

Comparison of the Molecular Dimensions on the Surface and in Solution. The paper is focused on the possibility to reconstruct the molecular structure in solution based on the visualized with AFM single molecules deposited on the mica surface. Thus, we study the rapidly deposited molecules in the state which is far from equilibrium conformation. Adsorption can strongly modify the conformation; however, the kinetics of the process can be relatively slow. At the same time, we may indeed expect changes in conformation of the molecules even during relatively short time of the contact with the substrate. In Table 5 we compare sizes of the single molecules evaluated with different methods in solution with the size of the shell obtained in the AFM experiments. The quite good agreement demonstrates that in this case the change of conformation on the surface is slow and the molecular size is not strongly changed during the deposition time.

Table 5. Comparison of the Molecular Radius (*R*) of Unimers in Solution and onto the Mica Surface after the Rapid Deposition

	radius, nm		
solvent	dynamic light scattering, R_h	gel permeation chromatography[a] R_{GPC}	AFM R_{AFM}
THF		15 (ref 17)	18 ± 5
toluene	31 (ref 9b)		40 ± 5
water, pH 2 (micelles)	85 (ref 9c)		97 ± 20

[a] Approximation.

The above-mentioned experiments provide a very nice example of how the interplay between different interactions at the solid–liquid interface may help to study behavior of polymer chain and how the interaction with substrate may help in single molecule experiment.

Conclusions

For the first time, we have directly counted the number of the P2VP second-generation arms of the heteroarm block/copolymer in the single molecule AFM experiment.

We found that PS_7–$P2VP_7$ block copolymers undergo diverse conformational transitions responding to external stimuli. At concentrations below 0.01 g/L, PS_7–$P2VP_7$ exists in molecularly dissolved state in both selective (acid water, toluene) and common good (chloroform, tetrahydrofuran) solvents. In the latter case the obtained structures on mica show that a “Janus”-like segregated structure is likely to exist in chloroform.

In acid conditions PS_7–$P2VP_7$ forms either unimolecular or multimolecular micelles depending on pH and ionic strength. PS_7–$P2VP_7$ undergoes the inverse intramolecular segregation upon addition of toluene. In this case, the inverse unimolecular micelles are constituted of the P2VP dense core and the PS swollen shell.

The PS core of unimers, adsorbed from acid water, *adapts* an extended conformation upon exposure to toluene whereas P2VP chains *retain* their extended conformation during this procedure. Thus, the obtained “squash” structures present a unique conformation which does not exist in any solvent and could not be obtained upon simple adsorption procedure.

Metallization of the PS_7–$P2VP_7$ improves AFM resolution due to the selective deposition of Pd clusters along the P2VP chains. Thus, the number of P2VP arms of the unimers as well as aggregation number of the multimolecular micelles can be directly estimated.

The conclusions from the most single molecule AFM experiments are in very good agreement with the results obtained from different methods exploring physical properties of molecule assembly. In our discussion we employed only few speculations. Finally, it gives evidence that, although the deposition of single molecule on a solid substrate may strongly alter the conformation in a general case, in the particular system the careful AFM experiment is indeed a powerful tool for the study of conformations of single polymer molecules.

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References and Notes

(1) Hadjichristidis, N.; Pispas, S.; Pitsikalis, M.; Iatrou, H.; Vlahos, C. *Adv. Polym. Sci.* **1999**, *142*, 71. Hadjichristidis, N.; Pitsikalis, M.; Pispas, S.; Iatrou, H. *Chem. Rev.* **2001**, *101*, 3747.

(2) Tsitsilianis, C.; Chaumont, P.; Rempp, P. *Makromol. Chem.* **1990**, *191*, 2319.

(3) Liu, S.; Armes, S. P. *Angew. Chem.* **2002**, *114*, 1413.

(4) Magonov, S. N.; Reneker, D. H. *Annu. Rev. Mater. Sci.* **1997**, *27*, 175. Sheiko, S. S.; Möller, M. *Chem. Rev.* **2001**, *101*, 4099. Tsukruk, V. V.; Reneker, D. H. *Polymer* **1995**, *36*, 1791.

(5) (a) Sheiko, S. S.; Möller, M. *Chem. Rev.* **2001**, *101*, 4099. (b) Shu, L.; Schlüter, A. D.; Ecker, C.; Severin, N.; Rabe, J. P. *Angew. Chem.* **2001**, *113*, 4802. (d) Gao, S.; Chi, L.; Lenhert, S.; Anczhykowski, B.; Niemeyer, C. M.; Adler, M.; Fuchs, H. *Chem. Phys. Chem* **2001**, *6*, 384. (c) Sheiko, S. S.; da Silva, M.; Shirvaniants, D.; LaRue, I.; Prokhorova, S.; Moeller, M.; Beers, K.; Matjaszewski, K. *J. Am. Chem. Soc.* **2003**, *125*, 6725. (d) Hansma, H. G.; Vesenka, J.; Siegerist, C.; Kelderman, G.; Morrett, H.; Sinsheimer, R. L.; Elings, V.; Bustamante, C.; Hansma, P. K. *Science* **1992**, *256*, 1180.

(6) Kumaki, J.; Hashimoto, T. *J. Am. Chem. Soc.* **2003**, *125*, 4907.

(7) (a) Minko, S.; Kiriy, A.; Gorodyska, G.; Stamm, M. *J. Am. Chem. Soc.* **2002**, *124*, 3218. (b) Kiriy, A.; Minko, S.; Gorodyska, A.; Stamm, M.; Jaeger, W. *Nano Lett.* **2002**, *2*, 881. (c) Kiriy, A.; Minko, S.; Gorodyska, A.; Stamm, M. *J. Am. Chem. Soc.* **2002**, *124*, 10192. (d) Kiriy, A.; Gorodyska, A.; Minko, S.; Jaeger, W., Štěpánek, P.; Stamm, M. *J. Am. Chem. Soc.* **2002**, *124*, 13454. (e) Gorodyska, A.; Kiriy, A.; Minko, S.; Tsitsilianis, C.; Stamm, M. *Nano Lett.* **2003**, *3*, 365.

(8) (a) Werts, M. P. L.; Van der Vegte, E. W.; Grayer, V.; Esselink, E.; Tsitsilianis, C.; Hadziioannou, G. *Adv. Mater.* **1998**, *10*, 452. (b) Grayer, V.; Dormidontova, E.; Hadziioannou, G.; Tsitsilianis, C. *Macromolecules* **2000**, *33*, 6330.

(9) (a) Voulgaris, D.; Tsitsilianis, C.; Esselink, F.; Hadziioannou, G. *Polymer* **1998**, *39*, 6429. (b) Voulgaris, D.; Tsitsilianis, C.; Grayer, V.; Esselink, F.; Hadziioannou, G. *Polymer* **1999**, *40*, 5879. (c) Tsitsilianis, C.; Voulgaris, D.; Štěpánek, M.; Podhájecká, K.; Procházka, K.; Tuzar, Z.; Brown, W. *Langmuir* **2000**, *16*, 6868.

(10) Tsitsilianis, C.; Voulgaris, D. *Macromol. Chem. Phys.* **1997**, *198*, 997.

(11) Xu, S.; Arnsdorf, M. F. *J. Microsc.* **1994**, *173*, 199. Vesenka, J.; Manne, S.; Giberson, R.; Marsh, T.; Henderson, E. *Biophys. J.* **1993**, *65*, 992.

(12) (a) Tsitsilianis, C.; Staikos, G.; Dondos, A.; Lutz, P.; Rempp, P.; Benoit, H. *Makromol. Chem*. **1990**, *191*, 2309. (b) Dondos, A.; Papanagopoulos, D. *J. Polym. Sci., Polym. Phys. Ed.* **1996**, *34*, 1281. (c) Tsitsilianis, C.; Kouli, O. *Macromol. Rapid Commun.* **1995**, *16*, 591.

(13) Minko, S.; Müller, M.; Usov, D.; Scholl, A.; Froeck, C.; Stamm, M. *Phys. Rev. Lett.* **2002**, *88*, 0355502.

(14) Brandrup, J.; Immergut, E. A.; Grulke, E. A. *Polymer Handbook*, 4th ed.; Wiley-Interscience: New York, 1999.

(15) Shull, K. R.; Kramer, E. J.; Hadziioannou, G.; Tang, W. *Macromolecules* **1990**, *23*, 4780.

(16) Wolterink, J.; Leermakers, F.; Fleer, G.; Koopal, L.; Zhulina, E.; Borisov, O. *Macromolecules* **1999**, *32*, 2365.

(17) Tsitsilianis, C.; Voulgaris, D. *Polymer* **2000**, *41*, 1607.

(18) Grayer, V.; Esselink, F. J.; Hadziioannou, G.; Tsitsilianis, C. 4th Panhellinio Conference on Polymers, Proceedings, 1997; p 267.

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