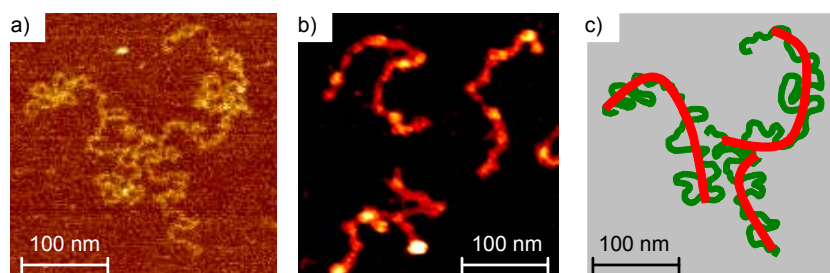


Single Polyelectrolyte Molecules: Visualization, Reconformation and Mineralization

Mittels Rasterkraftmikroskopie (AFM) wurden einzelne Moleküle des sternförmigen Blockcopolymers Poly(styren-block-2-vinylpyridin)-(PS₇-P2VP₇) untersucht. Dabei wurden Konformationsübergänge beobachtet, die durch verschiedene Lösungsmittel verursacht worden sind. Bei niedrigen Konzentrationen in selektiven Lösungsmitteln liegen die PS₇-P2VP₇ in molekular aufgelöstem Zustand vor und bilden unimolekulare Micellen. Die Zahl von P2VP-Armen des Unimers sowie die Aggregationszahl der multimolekularen Micellen und morphologische Details der Strukturen wurden direkt analysiert. Um die einzelnen Moleküle mittels AFM auch auf 'rauen' Substraten (Siliciumwafer) visualisieren zu können, wurde ein einfaches Kontrastierungsverfahren entwickelt. Es basiert auf Austausch der kleinen Cl⁻-Gegenionen durch große Hexacyanoferrat-Anionen oder negativ geladene Nanocluster des Preußischblaus. Die ursprüngliche Polymerkonformation wird durch diese Verfahren nicht geändert.

Introduction

The last decade has brought a revolution in the ability of chemists to study and manipulate single molecules. Such a progress is directly related with a development of the atomic force microscopy (AFM) which allows both visualization of single molecules and probing their properties [1]. In contrast to conventional physical and chemical methods which provide an information referred to ensemble of molecules, the AFM observation of individual members yields direct information about the distributions of their properties. Various important scientific and technological applications are connected with the visualization of single molecules: study of polymer's adsorption and re-conformation [2, 3], measurement of their molecular weight [4], performance of chemical reactions between single molecules [5], development of modern molecular electronics. The experiments were performed mainly with relatively thick 'exotic' polymeric molecules if the diameter of the chain was larger than 1 nm: dendronized polymers [5], 'molecular brushes' and some natural polymers [1]. However, most of synthetic polymers and many natural polymers have much thinner chains [6]. Recently, we succeeded to visualize several 'normal sized' polyelectrolytes (PE) in different conformations. These polymers can be imaged being deposited onto an atomically flat mica (root-mean square roughness, RMS less than 0.05 nm) but they are 'invisible' upon investigation on a silicon wafer (Si) due to slightly higher roughness of the surface ($RMS_{Si-wafer} = 0.2 \text{ nm}$) [2, 3].



Keywords

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Fig. 1: AFM topography images of P2VP molecules (a); P2VP metallized with Pd (b). Schematic representation of possible re-conformation of P2VP molecules (c) occurred upon the deposition of Pd: PE chains before (green) and after metallization (red).

Although several methods to improve the detection of polymer chains via chemical modification of tips or applying special AFM modes have been proposed [7], the development of techniques for study of *conformation of isolated chains with molecular resolution* on rough surfaces is still a challenging task.

Recently, we have described an approach to produce wire-shaped Pd nanoparticles via metallization of flexible synthetic PE [8, 9]. Metallized molecules are nicely resolved onto the Si-wafer with the tapping mode AFM and location of Pd clusters clearly reflects the location of underlying PE chains (Fig. 1b). Nevertheless, interaction of PE molecules with bivalent $[\text{PdCl}_4]^{2-}$ anion caused the strong contraction of polymer chains even if the chains were strongly trapped by the surface. Observed worm-shaped structures present a 'new' (distorted) conformations of PE, therefore this method can not be used for visualization of a 'true' (initial) conformation of PE molecules (Fig. 1c).

We developed the contrasting method for AFM to substantially improve the resolution of single molecule experiment virtually with no changes of the conformation of adsorbed polymer molecules. In our approach we use the deposition of either hexacyanoferrate $[\text{Fe}(\text{CN})_6]^{4-}$ anions or negatively charged clusters of cyanide-bridged complexes as contrasting agents. This method allowed us to increase the thickness of the resulting structures up to 10 nm, and, consequently, to provide visualization of polymer chains on Si-wafers and even usual glass slides with RMS = 1.5 nm [10].

Block copolymers composed of hydrophilic and hydrophobic segments form in aqueous media a micellar structure with a hydrophobic inner core and a hydrophilic outer shell. The micelles are widely used for stabilization of colloidal dispersions and in drug delivery systems because the lipophilic core serves as a 'microcontainer' for hydrophobic drugs. However, the micelles are unstable below a critical concentration that limits their possible applications. The star-shaped block copolymers in which hydrophobic and hydrophilic arms are connected together behave as unimolecular micelles in aqueous solution at any concentration. Using well-defined site-specific nano-environments within such unimers, in principle, it is possible to create various complicated entities promising for drug delivery, catalytic systems, electron and energy transfer units, or nanoelectronic devices.

Star-shaped block copolymers have attracted much interest for the last decades due to their unique complex architecture that introduces novel properties different from those of their linear counterparts. Such a molecule undergoes diverse conformational transitions in changing environment representing a very promising type of a responsive material for the fabrication of smart polymer films, micelles, and drug delivery systems.

Diverse Reformation of PS₇-P2VP₇ Star-Molecules

We performed the AFM study of star-shaped poly(styrene-*block*-2-vinylpyridine) (PS₇-P2VP₇, Figure 2a) heteroarm star copolymer as single molecule entities and showed the direct evidence of the molecular morphology and its response to controlled environment. At concentrations below 0.01 mg·ml⁻¹ PS₇-P2VP₇ exists in molecularly dissolved state in both selective (acidic water, toluene) and common good (chloroform, tetrahydrofuran) solvents. In acid conditions PS₇-P2VP₇ forms either unimolecular or multimolecular micelles depending on concentration and pH [11-13]. The core of the micelles is constituted of the collapsed PS arms surrounded by protonated P2VP shell (Figure 2b). PS₇-P2VP₇ undergoes inverse intramolecular segregation upon addition of toluene.

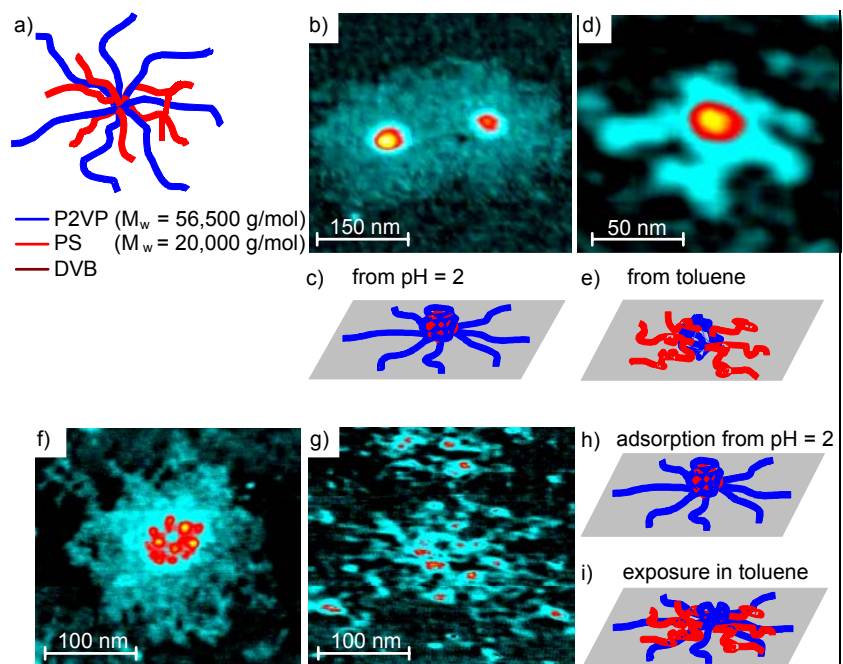


Fig. 2: Star shaped PS_7 - $P2VP_7$ block copolymer (a). AFM images and schematic representation of PS_7 - $P2VP_7$ unimers adsorbed on mica: from acidic aqueous solution (b-c); from toluene (d-e); adsorbed from acidic aqueous solution and then placed in toluene for 30 minutes (f); or for 5 hours (g). Schematic representation of the re-conformation (h-i)

In this case, P2VP arms form the dense core of the micelles embedded in the swollen PS shell (Fig. 2c). 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 *adopt 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 (Fig. 2 f-i) [13].

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 [8, 9]. 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 (Fig. 3).

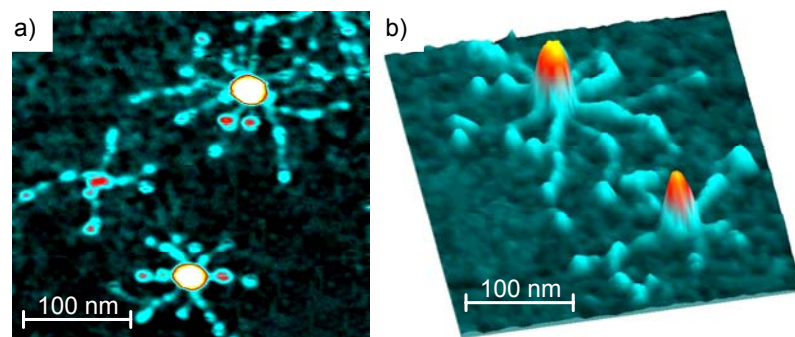
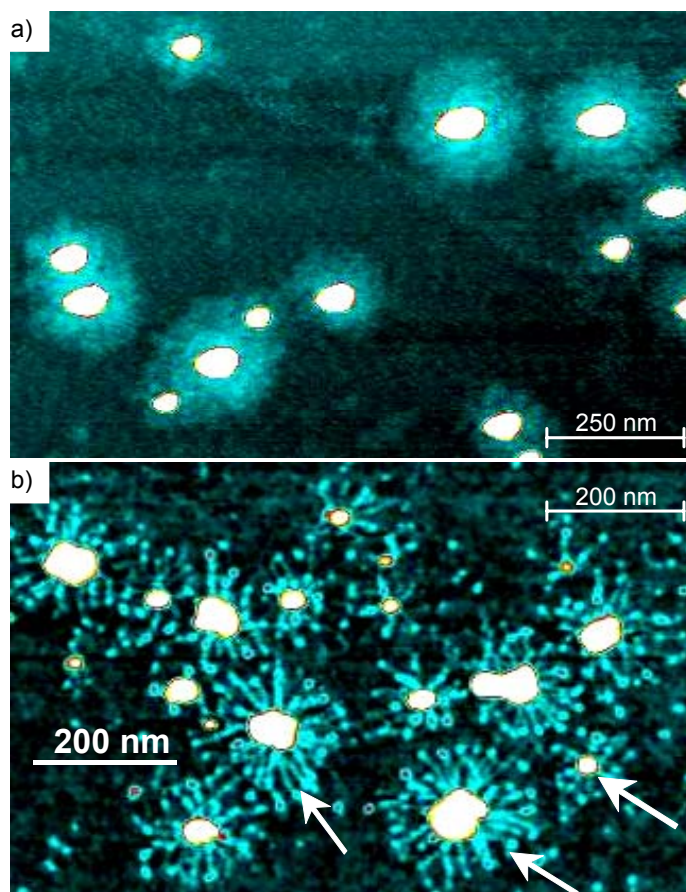


Fig. 3: 2D (a) and 3D AFM images of PS_7 - $P2VP_7$ unimers deposited from acidic aqueous and then metallized by Pd

The P2VP arms decorated with Pd clusters are clearly observed in the AFM images. 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 (Fig. 4).

Fig. 4:
Coexistence of uni- and multi-molecular micelles at concentration of $PS_7\text{-}P2VP_7$ $0.03 \text{ g}\cdot\text{l}^{-1}$ (HCl, $\text{pH} = 1$) before (a) and after metallization with Pd (b). Metallization improves AFM contrast and thus aggregation number can be directly estimated. For example, the structure marked by the yellow arrow corresponds to tetramer with about 28 P2VP arms, by the green arrow to trimer with about 21 P2VP arms, and the red arrow shows the unimer with 7 P2VP arms.



Conditions	Shell		Core	
	D [nm]	H [nm]	D [nm]	H [nm]
CHCl_3^{**}	46 ± 8	1 ± 0.2	18 ± 4	2.6 ± 0.2
THF**	36 ± 10	2.4 ± 0.5		
$\text{pH} = 2^{**}$	126 ± 35	0.3 ± 0.1	17 ± 7	2.9 ± 0.3
Pd-unimers	122 ± 30	1 ± 0.2	9 ± 2	7.2 ± 2
$\text{pH} = 2, \text{Na}_3\text{PO}_4$	99 ± 15	0.1 ± 0.2	15 ± 5	5.6 ± 0.5
$\text{pH} = 3.5$ (81 % unimers, 19 % micelles)	119 ± 30	0.3 ± 0.1	10 ± 2 20 ± 4	4.9 ± 0.5 16 ± 3
$\text{pH} = 4.2$ (micelles)**	137 ± 35	0.3 ± 0.1	22 ± 5	17 ± 1
$\text{pH} = 1, 0.3 \text{ g}\cdot\text{l}^{-1} PS_7\text{-}P2VP_7$ (micelles)	–	–	34 ± 5	15.9 ± 3
$\text{pH} = 1, 0.1 \text{ g}\cdot\text{l}^{-1} PS_7\text{-}P2VP_7$ (micelles)	–	–	30 ± 5	12.5 ± 3
$\text{pH} = 1, 0.03 \text{ g}\cdot\text{l}^{-1} PS_7\text{-}P2VP_7$ (micell.)	195 ± 40	0.4 ± 0.1	25 ± 5	12.1 ± 3
Pd-multi-micelles	151 ± 50	1 ± 0.2	40 ± 10	25.4 ± 5
Toluene 30min	80 ± 10	0.5 ± 0.2	36 ± 10	2.4 ± 0.3
$\text{pH} = 2$, then toluene	129 ± 40	0.4 ± 0.1	41 ± 10	2.3 ± 0.5

Table 1:
Average dimensions of the $PS_7\text{-}P2VP_7$ (on the basis of at least 100 structures taken from 5-10 AFM images for each conditions).

The AFM images for the first time visualize the second generation of the P2VP arms, which have grown 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 , that was in excellent agreement with the number of PS arms found by light scattering. Table 1 summarize dimensions of structures formed from PS₇-P2VP₇ at different conditions [13].

Chemical Contrasting in Single Molecule AFM Experiment

Both poly(methacryloyloxyethyl dimethylbenzyl ammonium chloride) (PMB) and P2VP molecules deposited onto Si-wafer are not resolved in the tapping mode (Fig. 5b). Although PS₇-P2VP₇ molecules adsorbed onto mica from acid water (pH = 2) solution display a clear core-shell morphology (Fig. 5e), only the core of unimers with the height of 5 nm can be resolved on the Si-wafer (Fig. 5f). We assumed that selective deposition of appropriate materials along polyelectrolyte (PE) molecules (such as bulky hexacyanoferrate counterions or Prussian Blue (PB) nanoclusters) could be a very promising method to increase the thickness of the PE chains and thus make them well-recognizable even on rough surfaces (Fig. 5a). Fig. 5c presents AFM images of PMB adsorbed onto the Si-wafer and then stained with HCF acid solution (pH 2, HCl). We observe an increase of heights of resulting structures approximately on 6-7 Å which roughly corresponds to the dimension of HCF-anion.

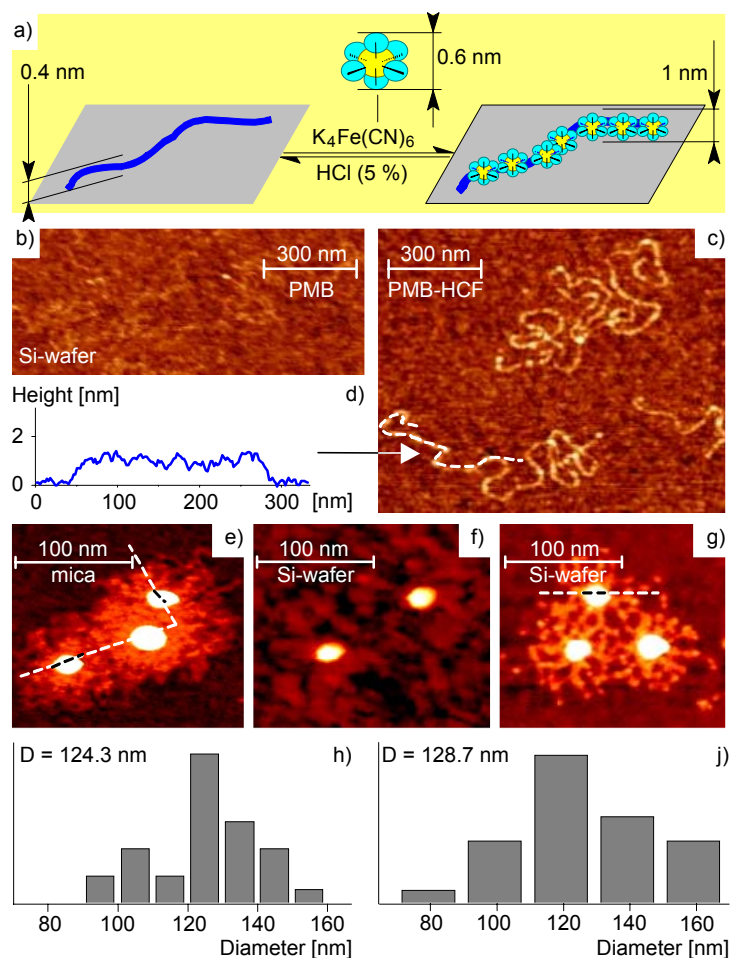


Fig. 5: Scheme of the contrasting of adsorbed polycations (a), AFM topography images (b, c) and a cross-section (d) of PMB, and AFM images of PS₇-P2VP₇ molecules before (b, e, f), and after (c, g) contrasting with HCF (Z-range 5 nm). Histograms of molecular diameter distribution for PS₇-P2VP₇ adsorbed onto the mica, no contrasting (h), and onto the Si-wafer after contrasting with HCF (j).

The statistical analysis of the molecular diameter from AFM images of the PS₇-P2VP₇ unimers before the contrasting on mica and after the contrasting on the Si-wafer provides evidence that the contrasting procedure introduces no changes in the conformation (Fig. 5h, j). Similarly, we detected no changes of the dimensions of PMB molecules upon staining with K₄Fe(CN)₆.

We performed a special experiment to precisely monitor the effect of the staining on molecular details of the PC conformations deposited onto the lithography-patterned Si-wafer with a relatively high roughness (0.5 nm) Si-wafer by scanning always one place and visualizing the same molecules before and after the contrasting. Ultra-fast spin-coating was applied to deposit PMB molecules in stretched conformation. As a representative example, Figure 5 shows images of several PMB molecules taken before and after the staining with K₄Fe(CN)₆. Although 'as deposited' PMB molecules are poorly resolved on AFM images (Fig. 6a, b, e), they are clearly observed after the contrasting with K₄Fe(CN)₆ (Fig. 6c, d, f). Long molecules (**A** and **D**) and short chains (for example, **B**) adopt stretched conformation and their contour length can be easily measured: $L_A = 3339$ nm; $L_B = 275$ nm; $L_D = 2727$ nm. Although contour length of several molecules (marked 'C' in Figure 6), cannot be measured due to the strongly coiled conformation, the detailed analysis of the AFM data clearly indicates that increase of the chain thickness from a few Ångstrom to 1.5 nm (Fig. 6g, h) upon treatment with K₄Fe(CN)₆ occurs virtually without any changes in their location, contour length and fine details of the conformation within the resolution limit.

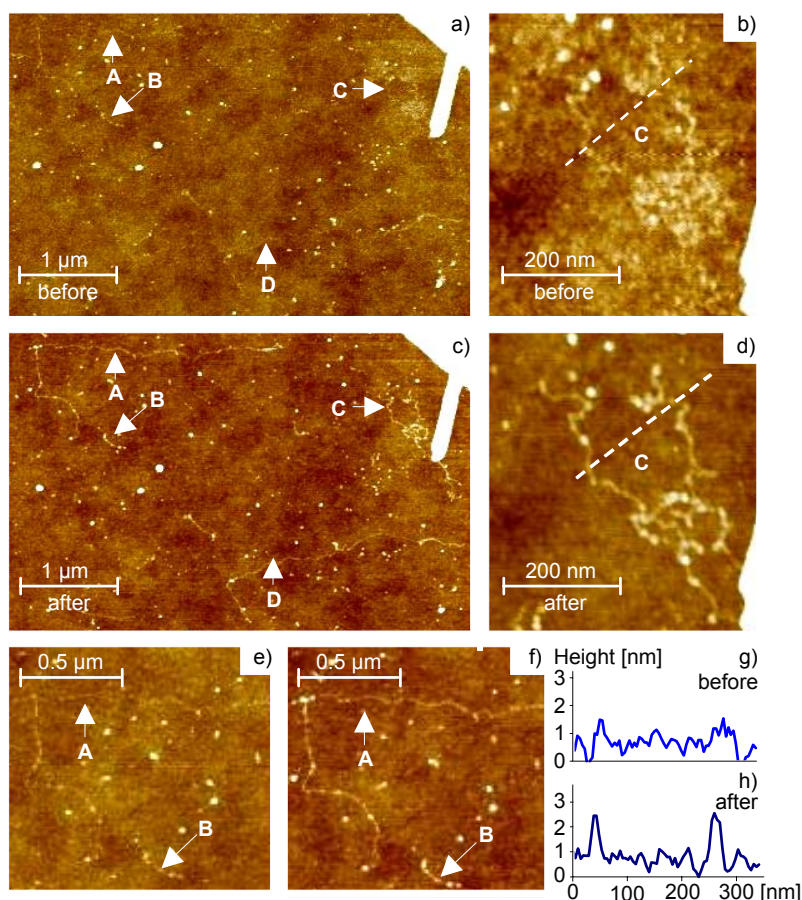


Fig. 6: AFM topography images of the PMB molecule deposited on the lithography-patterned Si-wafer before (a, b, e), and after (c, d, f) contrasting with HCF (Z-range 5 nm); g and h are cross-sections of images b and d, respectively, cross-sections are taken at the same place of the Si-wafer before and after the contrasting reflect 1 nm increase of the height of PMB molecules. Green letters **A-D** mark several PMB molecules distinguished onto the surface.

We have also found that minor part of molecules appears to be partially distorted during the procedure. The transformation of the molecule **D** is shown in Figure 7.

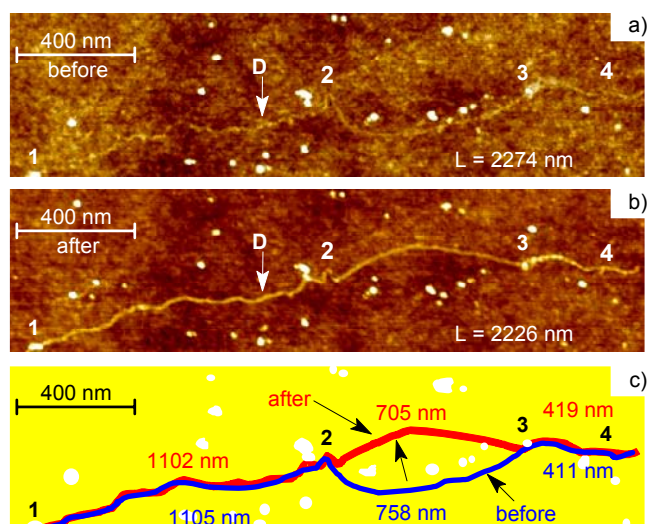


Fig. 7: AFM topography images of the PMB molecule on Si-wafer before (a), and after (b) contrasting with HCF (Z-range 5nm). Snapshot (c) demonstrates the transition of the 2-3 segment induced by the contrasting procedure (see explanations in the text).

The 2.2 μm long PMB molecule **D** deposited on the Si-wafer in the extended conformation is visualized before (Fig. 7a) and after (Fig. 7b) staining. After the contrasting with $\text{K}_4\text{Fe}(\text{CN})_6$ it is thicker and nicely visible. Although the segments **1-2** and **3-4** of the molecule with the length of 1100 nm and 410 nm, respectively, remained unchanged, the central part **2-3** with the initial length of 758 nm has been moved up for about 250 nm, as it is shown by the green arrow on the snapshot (Fig. 7c). The transformation is accompanied with about 7 % collapse of the **2-3** fragment. Thus, we see the important role of the attractive interaction between the surface and PC molecule. If a PMB chain (or its fragment) bearing $[\text{Fe}(\text{CN})_6]^{4-}$ anions loses the contact with the surface it collapses due to the counterion condensation effect [10].

We have found that deposited HCF can be removed without changes of the molecular dimensions of PC simply upon rinsing the sample with either acid (HCl, 5 %) or basic (NH_3 , 3 %) water solution for several minutes.

Deposition of Prussian Blue Nanoclusters

It is well known that mixing of stoichiometric amounts of $\text{K}_4\text{Fe}(\text{CN})_6$ and FeCl_3 leads to microscopic particles of PB ($\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$) which precipitate from solution. Several methods to fabricate well-defined surfactant- or polymer-protected PB nanoclusters have been recently reported. We developed a simple method to produce surfactant-free water-soluble negatively charged PB nanoclusters stabilized by excess of HCF-anions. Specifically, we found out that mixing of diluted solution of FeCl_3 and with excess of $\text{K}_4\text{Fe}(\text{CN})_6$ solution in acid conditions ($\text{pH} = 2$) leads to clear deep-blue dispersions stable during several months.

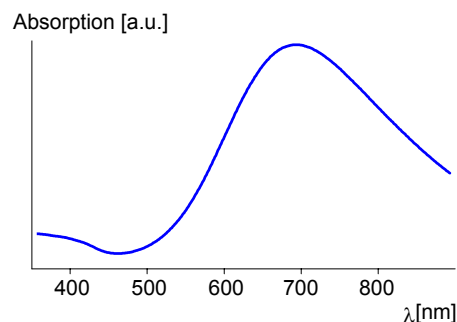


Fig. 8: UV-vis spectra of Prussian Blue dispersion (prepared at molar ratio $[\text{K}_4\text{Fe}(\text{CN})_6]:[\text{FeCl}_3] = 4:1$, PB-2), λ = wavelength.

The characteristic blue color and broad signal in the UV-vis spectra with λ_{\max} at 695 nm are consistent with an intermetal charge-transfer band from Fe^{2+} to Fe^{3+} and reflect the formation of PB (Fig. 8). In all cases PB clusters display narrow distributed size (PDI = 1.1-1.2) which depends on $[\text{K}_4\text{Fe}(\text{CN})_6]:[\text{FeCl}_3]$ mixing ratio (Fig. 9). We have found that stabilization of the size of PB clusters occurs within 10 min.: mixing of $\text{K}_4\text{Fe}(\text{CN})_6$ and FeCl_3 solutions at molar ratio of 8:1 leads to PB particles at the diameter of about 2.7 nm (PB-1, Fig. 9 a-d) whereas mixing at molar ratio of 4:1 leads to the PB-2 clusters with the diameter near 4.8 nm (Fig. 9 e-h).

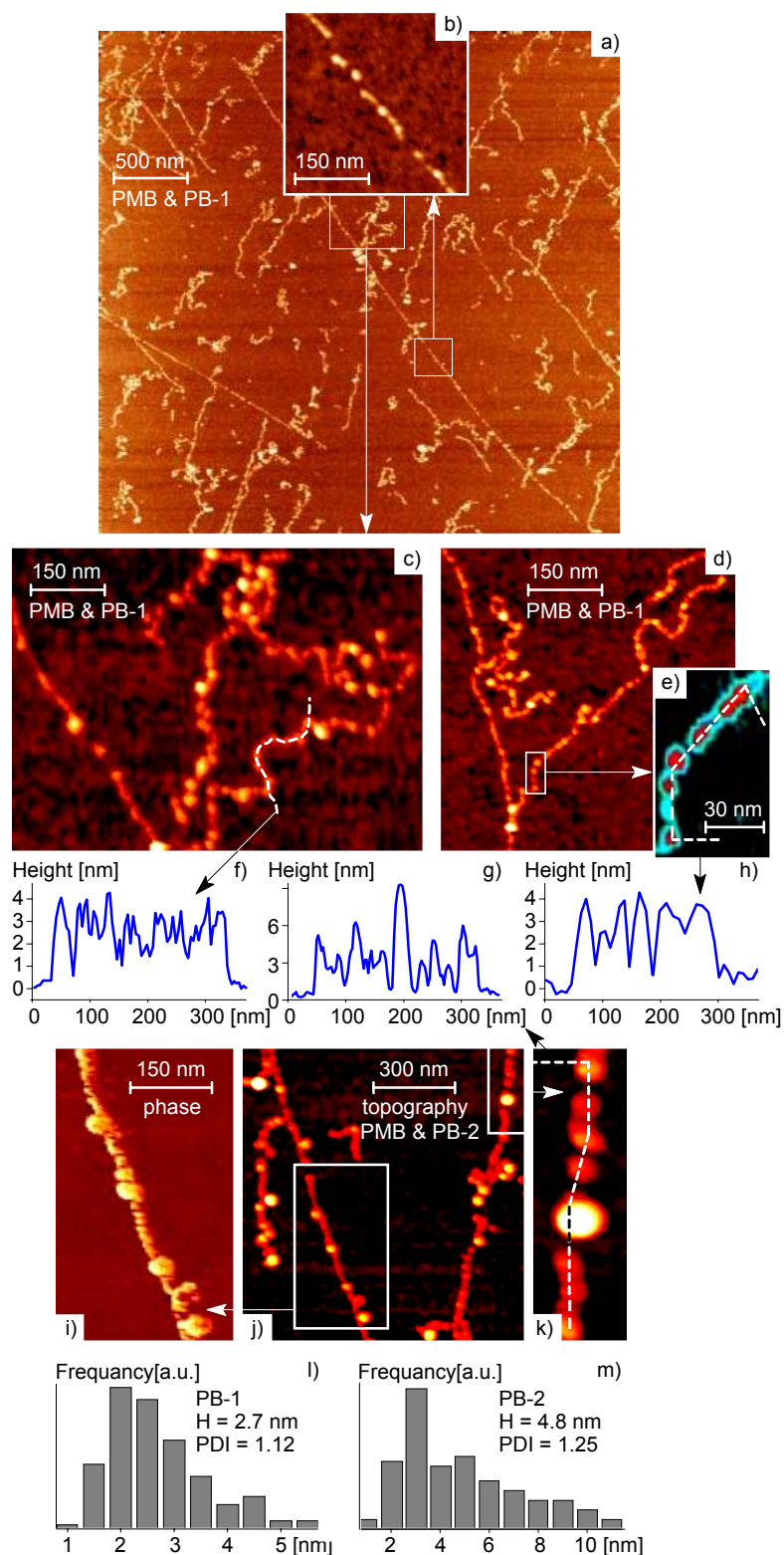


Fig. 9: AFM phase (i) and topography (all other, Z-range 10 nm) images of the PMB molecules deposited onto the Si-wafer by the spin-stretching and then contrasted with PB nanoparticles (prepared at molar ratio $[\text{K}_4\text{Fe}(\text{CN})_6]:[\text{FeCl}_3] = 8:1$, PB-1 for images (a-e) and 4:1, PB-2 for images (i-k)). The dash line in (c, e, k) indicates the locus of the cross-sections (f, h, g, respectively). Histogram (l, m) present the size distribution of the PB clusters.

Using these dispersions in the contrasting procedure results in the decoration of PC with the PB clusters. The product appears as a structure of beads-on-string morphology (Fig. 9). The attachment of PB clusters significantly improves both the topography and phase AFM images (Fig. 9i). Rather high binding density of PB clusters (the distance between adjacent clusters is about 15 nm for PB-1, Fig. 9h and 30 nm for PB2, Fig. 9g) is sufficient for the visualization of the molecular details of PC conformations on surfaces with the RMS larger than 0.5 nm.

Nevertheless, as seen from zoom-image (Fig. 9b) some of stretched PMB chains contain 10-30 nm gaps. It is important, these gaps remain unchanged even after repeating of the staining procedure. Such a result reflects a fragmentation process occurred upon the binding of PB clusters.

To demonstrate the ultimate ability of the method we contracted PMB chains deposited onto the microscope glass slides with RMS about 0.8 nm. Despite the size of the surface features and diameter of PB-3 clusters are comparable (3-7 nm) after the contrasting PMB chains can be easily recognized (Fig. 10).

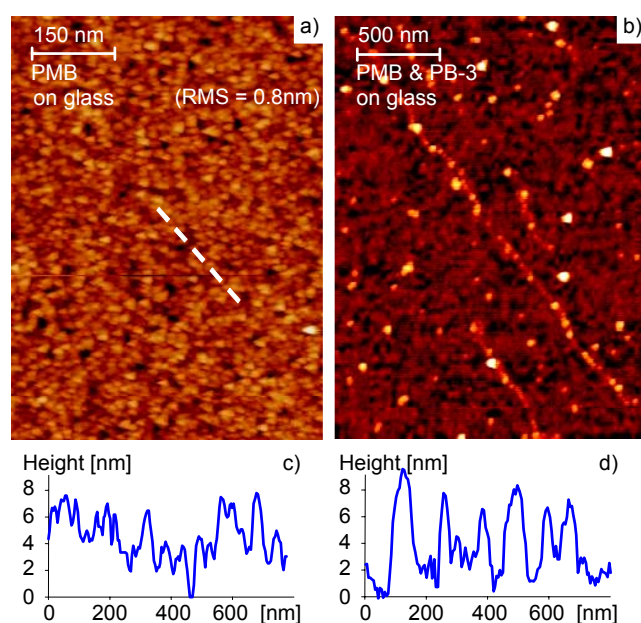


Fig. 10: AFM topography images (Z-range 15 nm) and cross-sections (c, d) of the PMB molecules deposited onto the microscope glass slide before (a, c) and after (b, d) the contrasting with PB-3 clusters (prepared at molar ratio $[K_4Fe(CN)_6]:[FeCl_3] = 4:1$).

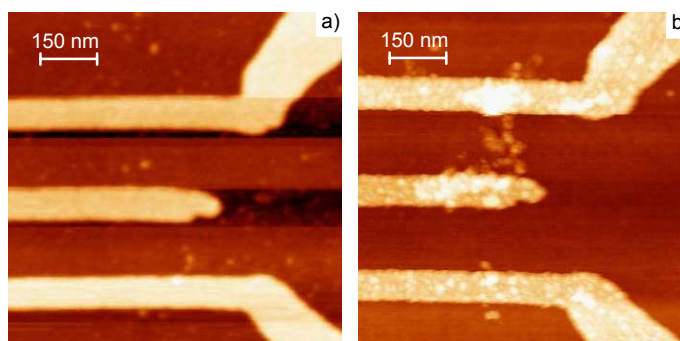
Conclusions and Outlook

Single synthetic polyelectrolytes molecules of various architecture and composition have been visualized in different conformation after deposition onto the mica surface. We have found that PS₇-P2VP₇ block copolymers undergo diverse conformational transitions responding to external stimuli. At concentrations below 0.01g·l⁻¹, PS₇-P2VP₇ exists in molecularly dissolved state in both selective (acid water, toluene) and common good (chloroform, tetrahydrofuran) solvents. In acid conditions PS₇-P2VP₇ forms either unimolecular or multimolecular micelles depending on pH and ionic strength. PS₇-P2VP₇ 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.

Metallization of the PS₇-P2VP₇ 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 the aggregation number of the multimolecular micelles can be directly estimated.

We have developed a simple contrasting procedure to improve the AFM visualization of positively charged polymer chains deposited on the substrates of relatively high roughness via counterion exchange between Cl^- anions and bulky $[\text{Fe}(\text{CN})_6]^{4-}$ anions or negatively charged nanoclusters of Prussian Blue. We believe that our innovation is important for the development of single molecule experiments with polymer chains. The reaction of HCF-anion could be used for recognition of polycation molecules, when polycations, polyanions and neutral molecules coexist on the surface. Recently, the study was strongly restricted to atomically smooth surfaces. The contrasting procedure extends the range of substrates (Si-wafers, chemically modified or patterned Si-wafers, polished glasses, polymer films, etc) appropriate for the experiments. That is very useful for the study of numerous processes involving the interaction of synthetic and biomacromolecules with various surfaces. Such a progress could be a basis for further development of nanoscale electronics. In the frame of the *International Eurocores SONS Project* started in October 2003 we will use the developed approaches for fabrication of various simple elements and nanodevices: conductive wires, molecule based field-effect transistors, and photo-resistors.

Fig. 11:
AFM images depict our attempt to produce conductive nanowire from PE molecule: bare lithography-made nanoelectrodes (a); the same place of the electrode after the stretching of PE molecule followed by metallization with Pd (b).



We will place PE molecule between lithography-made nanoelectrodes, for example, as shown in Fig. 11, and then we will deposit desired active materials selectively along the molecule.

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