

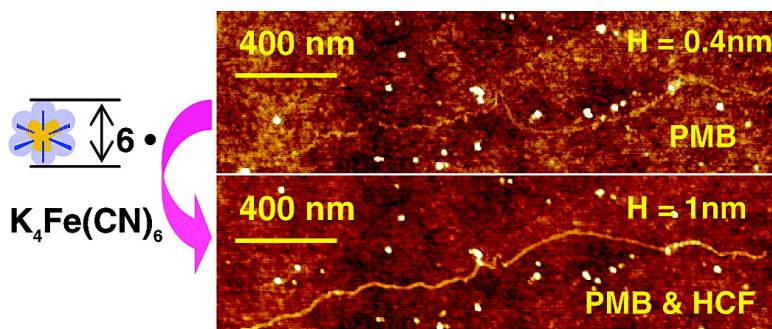
Communication

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## Chemical Contrasting in a Single Polymer Molecule AFM Experiment

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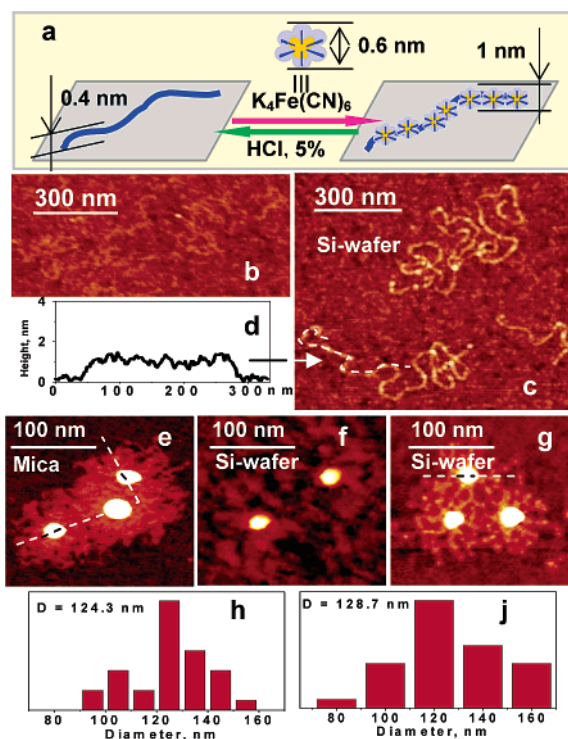
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Atomic force microscopy (AFM) is used for the visualization of single polymer molecules and the probing of their properties.<sup>1</sup> New knowledge was obtained from the AFM study of polymer adsorption and reformation in a controlled environment,<sup>2</sup> estimation of molecular weight,<sup>3</sup> and monitoring of chemical reactions between single polymer molecules.<sup>4</sup> The experiments were performed mainly with relatively “thick” polymeric molecules if the diameter of the chain was larger than 1 nm: dendronized polymers,<sup>4</sup> “molecular brushes”, and some naturally occurring polymers.<sup>1</sup> However, most of the synthetic polymers and many natural polymers have much thinner chains.<sup>5</sup> Although several methods to improve the detection of polymer chains via chemical modification of tips or applying special AFM modes have been proposed,<sup>1f,g</sup> the development of techniques for the study of the conformation of isolated chains with molecular resolution on rough surfaces is still a challenging task. Recently, we succeeded in visualizing polycations (PC) in different conformations adsorbed onto the atomically flat mica.<sup>2</sup> Although these PC are invisible on a Si-wafer due to the high roughness of the substrate,<sup>6</sup> they can be resolved after the decoration of the deposited chains with Pd clusters.<sup>7</sup> Metallization of PC causes the strong contraction of chains (2–3 times decrease of the contour length) even if the chains were strongly trapped by the substrate. The local collapse of the chain was induced by interaction with the bivalent ion PdCl<sub>4</sub><sup>2-</sup>.

Here, we report for the first time on the contrasting method for AFM to substantially improve the resolution of a single molecule experiment with no changes of the conformation of adsorbed polymer molecules. In our approach, we use the deposition of either hexacyanoferrate (HCF) anions or negatively charged clusters of cyanide-bridged complexes as contrasting agents (Figure 1a). This method allowed us to increase the thickness of the resulting structures up to 3 nm and, consequently, to provide visualization of polymer chains on Si-wafers. After AFM-measurements, the contrasting agents were then removed also without distortion of the molecule conformation.

We used in this study three different PC molecules: poly(methacryloyloxyethyl dimethylbenzylammonium chloride) (PMB)<sup>2a</sup>  $M_w = 6130$  kg/mol, poly(2-vinylpyridine) (P2VP)<sup>2b</sup>  $M_w = 385$  kg/mol, and the star-shaped heteroarm block copolymer with seven polystyrene  $M_w = 20$  kg/mol and seven P2VP  $M_w = 56.5$  kg/mol arms (PS<sub>7</sub>-P2VP<sub>7</sub>).<sup>2c</sup> Both PMB and P2VP molecules deposited onto the Si-wafer are not resolved in the tapping mode (Figure 1b).<sup>8</sup> Although PS<sub>7</sub>-P2VP<sub>7</sub> molecules adsorbed onto mica from acid water (pH 2) solution display a clear core–shell morphology (Figure 1e),<sup>2c</sup> only the core of unimers with the height of 5 nm can be resolved on the Si-wafer (Figure 1f). We were successful in visualizing all



**Figure 1.** Scheme of the contrasting of adsorbed polycations (a), AFM topography images (b,c), and a cross-section (d) of PMB, and AFM images of PS<sub>7</sub>-P2VP<sub>7</sub> molecules before (b,e,f) and after (c,g) contrasting with HCF (Z-range 5 nm). All images are on Si-wafers, but (e) is on mica. Histograms of molecular diameter distribution for PS<sub>7</sub>-P2VP<sub>7</sub> adsorbed onto the mica, with no contrasting, are shown in (h), and those onto the Si-wafer after contrasting with HCF are shown in (j).

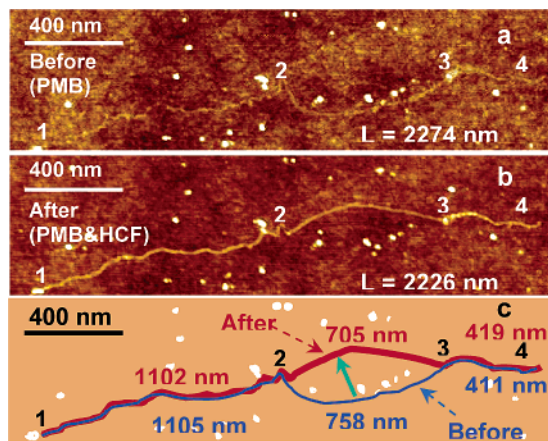
of these polymers on the Si-wafer substrate with the following staining procedure. The samples were prepared as described elsewhere.<sup>2,7</sup> The polymer molecules were deposited on the freshly cleaved mica or Si-wafers from a very diluted (0.0005 mg/mL) acid solution (pH 2.5–3). The drop of the examining solution was set on the substrate for 60 s, and afterward it was removed with a centrifugal force. The molecules were examined in a dry state with a Multimode AFM instrument (Digital Instruments, Santa Barbara) in the “light” tapping mode. Details of the AFM experiments were reported elsewhere.<sup>2,7</sup> The samples were then stained upon exposure for 3 min to an HCF acid solution bath. The sample was rinsed in water and dried for the AFM experiment. Figure 1c,g presents AFM images of PMB and PS<sub>7</sub>-P2VP<sub>7</sub> molecules contrasted with HCF. In all cases, we observed an 0.6–0.7 nm increase of heights of resulting structures,<sup>9</sup> that roughly corresponded to the size of the HCF-anion (Figure 1a). We found a strong effect of pH on the contrasting process. No attachment of HCF-anions was observed at pH higher than 4, that is, above the isoelectric point of the Si-

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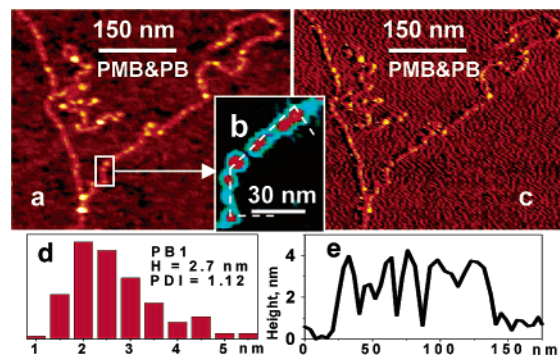


**Figure 2.** AFM topography images of the PMB molecule on the Si-wafer (RMS =  $0.6 \pm 0.4$  nm) before (a) and after (b) contrasting with HCF (Z-range 5 nm). Snapshot (c) demonstrates the transition of the 2–3 segment induced by the contrasting procedure (see explanations in the text).

wafer (pH 3.8). We may speculate that at the large pH the negatively charged Si-wafer suppresses the interaction of the polycations with HCF-anions. The statistical analysis of the molecular diameter from AFM images of the PS<sub>7</sub>-P2VP<sub>7</sub> 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 (Figure 1h,j). Similarly, we detected no changes of the dimensions of PMB molecules upon staining with HCF. We found that deposited HCF can be removed without changes of the molecular dimensions of PC simply upon rinsing the sample with either acidic (HCl, 5%) or basic (NH<sub>3</sub>, 3%) water solution for several minutes.

We observed precisely the effect of the staining on the molecular details of the PC conformations for the molecules deposited on the Si-wafer by scanning always the same place (a place we found using a label on the surface of the Si-wafer prepared with lithography) and visualizing the one molecule before and after the contrasting. The detailed analysis of the AFM data proved that the location and fine conformational details of most of the PMB molecules remain unchanged upon treatment with HCF. We also found that a minor part of the molecules appears to be partially distorted during the procedure. A “negative” example is shown in Figure 2. The 2.2  $\mu$ m long PMB molecule deposited on the Si-wafer in the extended conformation is visualized before (Figure 2a) and after (Figure 2b) staining. After the contrasting with HCF, it is thicker and nicely visible. Although the segments 1–2 and 3–4 of the molecule with lengths of 1100 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 (Figure 2c). The transformation is accompanied with about a 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 HCF-anions loses contact with the surface, it collapses due to the counterion condensation effect.

With the contrasting procedure, the size (thickness) of the chain can be even more increased by the attachment of larger counterions or even negatively charged clusters. For that we used the clusters of Prussian blue (PB) (Figure 3). We found out that addition of FeCl<sub>3</sub> to 6 molar excesses of HCF results in the dispersion of the PB nanoclusters of narrow size (2.7 nm averaged diameter) distribution (Figure 3d). Obviously, the stability of the PB clusters is due to the high negative charge provided by the excess of HCF-anions located on the clusters’ surfaces. Using this dispersion in



**Figure 3.** AFM topography (a,b) (Z-range 10 nm), phase (c) images, and cross-section (e) of PMB contrasted with PB nanoparticles. The dash line in (b) indicates the locus of the cross-section (e). Histogram (d) presents the size distribution of the PB clusters.

the contrasting procedure results in the decoration of PC with the PB clusters. The product appears (Figure 3b) as a structure of the beads-on-string morphology. The attachment of PB clusters significantly improves both the topography (Figure 3a,b) and the phase AFM images (Figure 3c). The high binding density of PB clusters (the distance between adjacent clusters is about 15 nm) is sufficient for the visualization of the molecular details of PC conformations on surfaces with an RMS larger than 0.5 nm.

In summary, we developed the 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<sup>−</sup> anions and bulky HCF 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.

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- (9) In the reference experiment, we found no adsorption of K<sub>4</sub>Fe(CN)<sub>6</sub> onto bare substrates (without preadsorbed PC molecules) either at neutral pH or in acid conditions, as confirmed by AFM and ellipsometry.

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