

Investigation on the Folding Mode of a Polymer Chain in a Spiral Crystal by Single Molecule Force Spectroscopy^{*}

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Abstract Investigation on the folding mode of a single polymer chain in its crystal is significant to the understanding of the mechanism of the fundamental crystallization as well as the engineering of new polymer crystal-based materials. Herein, we use the combined techniques of atomic force microscopy (AFM) imaging and force spectroscopy to pull a single polyethylene oxide (PEO) chain out of its spiral crystal in amyl acetate. From these data, the folding mode of polymer chains in the spiral crystal has been reconstructed. We find that the stems tilt in the typical flat area, leading to the decrease in the apparent lamellar height. While in the area of screw dislocation, the lamellar height gradually increases in the range of several nanometers. These results indicate that the combined techniques present a novel tool to directly unravel the chain folding mode of spiral crystals at single-molecule level.

Keywords: Folding mode; Spiral crystal; Single molecule force spectroscopy; Atomic force microscopy.

INTRODUCTION

Understanding the folding mode of polymer chains in the spiral crystal will shed light on the mechanism of screw dislocation during crystallization^[1]. The introduction of novel techniques, such as *in situ* atomic force microscopy, modulated and high speed calorimetry, microbeam X-ray scattering, combination of standard techniques in simultaneous measurements, or the use of new evaluation procedures in scattering experiments, has expanded the knowledge on the lamellar twist, greatly^[2–6]. Nevertheless, the folding mode within the spiral crystal is still not completely understood due to the relatively small amount of clear-cut experimental verification at single-molecule level.

Single molecule force spectroscopy (SMFS) has become a powerful tool during the past decades with the development of AFM^[7, 8], optical tweezers^[9], magnetic tweezers^[10], bio-membrane force probe and other single-molecule manipulation techniques. Those methods have enabled numerous single-molecule mechanical researches in material and life sciences^[11–13]. Due to the fact that some force spectra can be correlated well with the force-induced conformational change of single polymer chain, the force spectrum can also be termed as force fingerprint of the specific polymer system, such as the saw-tooth pattern may represent the unfolding of multiple polyproteins^[11, 14], the sequential unbinding of RNA-protein complexes^[15] or desorption of multiple polymer

^{*} This work was financially supported by the National Natural Science Foundation of China (Nos. 20974039, 21221063 and 91127031), the National Basic Research Program (2013CB834503), the Program for New Century Excellent Talents in Universities (NCET-11-0205).

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Invited paper for the special issue of “Polymer Crystallization”

Received March 10, 2014; Revised April 24, 2014; Accepted May 9, 2014

doi: 10.1007/s10118-014-1501-2

loops from the substrate^[13]; long plateau-containing force curves may reflect the unfolding of the helical structures^[16, 17] or detachment of train-like polymer fragments from the substrate^[13, 18].

In this study, we use AFM-based single molecule force spectroscopy to pull an individual PEO molecule out of its spiral single crystal to directly establish the relationship between nanomechanical properties and folding mode at single-molecule level. Single-molecule experiments were performed in typical flat area and the area of screw dislocation in spiral crystal. In addition, SMFS results on PEO spiral crystals were compared with those in normal single crystal, and different folding modes were identified for spiral crystal. These results have deepened our understanding on the molecular structure of spiral crystals, and may be useful for establishing the theoretical model of the formation mechanism of spiral crystals.

EXPERIMENTAL

Materials

Thiol-terminated polyethylene oxide (HS-PEO-OCH₃, 48.5 kg/mol) was purchased from Polymer Source Inc. Amyl acetate, tetraoctylammonium bromide (TOAB), dimethylsulfoxide (DMSO), *N*-(α -maleimidoacetoxy) succinimide ester (AMAS) and molecular sieve were purchased from Aldrich. Hexanedithiol was purchased from Alfa. Potassium borohydride, H₂AuCl₄·4H₂O and sodium citrate were purchased from GuoYao. 3-Aminopropyltrimethoxysilane was purchased from Fluorochem, UK. All materials were used as received.

Preparation of PEO Spiral Crystals

PEO spiral crystals were obtained by self-seeding method. Briefly, 1 mg PEO was dissolved in 10 mL amyl acetate at 60 °C for 10 min. The hot solution was then transferred to a water bath of rather low temperature (5 °C) for 4 h. Then the polymer solution was heated to the seeding temperature (46 °C) with a heating rate of 1 K/min and held at this temperature for 10 min to form stable and uniform nuclei. Subsequently, the solution was brought to the crystallization temperature (38 °C) and crystallized for 30 min. The suspension of PEO single crystals were then deposited on to AMAS-modified glass surface and incubated for 10 min, after that the sample was rinsed with amyl acetate.

Preparation of 5 nm Gold Nanoparticles

1 mL of 1% H₂AuCl₄ and 2 mL of 0.03 mol/L sodium citrate were added to 50 mL of purified water with vigorously stirring. Then, 1 mL of 0.1 mol/L KBH₄ was added. After stirring, the solution was left undisturbed for 2 h. The average diameter of the nanoparticle was about 5 nm as measured by AFM.

Immobilization of 5 nm Nanoparticles on HS-PEO Spiral Crystal

1 g of 0.5% TOAB/amyl acetate was mixed with 2 g nanoparticle (5 nm). The mixture was vigorously stirred and centrifuged for 10 min at 2000 r/min. Then, the organic phase was collected. Molecular sieve was used to remove the residual water. HS-PEO spiral crystals were directly deposited on AMAS-modified glass wafers. The TOAB-protected nanoparticles dispersed in amyl acetate were dropped on the HS-PEO spiral crystal modified glass surface and incubated for 1 h. After that, the fresh amyl acetate was used to wash away the free nanoparticles^[19].

AFM Measurements and Single Molecule Force Spectroscopy

All experiments were performed on a NanoWizard II BioAFM (JPK Instrument AG, Berlin, Germany). For force spectroscopy experiments, the spring constants of AFM cantilevers were calibrated in solution using the thermal noise method to obtain the spring constant (typically around 60 pN/nm and 1000 pN/nm (for the indentation and penetration experiment) for Si₃N₄ cantilevers from Bruker Corp.; 30 pN/nm for Bio-lever, Olympus Japan)^[20]. All experiments were carried out in amyl acetate at room temperature.

RESULTS AND DISCUSSION

Folding Mode of a Single PEO Chain in Typical Flat Area of Spiral Crystal

To detect the folding modes of polymer chains in their crystals, we picked them up individually from different

areas of the crystal surfaces (Figs. 1a and 1b) with the AFM tip as described in our previous study^[1, 21]. In brief, thiol labeled PEO spiral crystal was immobilized by one side on glass substrate, leaving the other side of the crystal surface exposed for being picked up by the gold-coated AFM tip. Under the guidance of AFM imaging, the AFM tip is located in (and contacted with) the specific area on the crystal surface, in which way the thiol end of the polymer chain may get attached onto the AFM tip *via* gold-thiol chemistry; and then the corresponding PEO chain could subsequently be pulled out of its single crystal during the retraction of the AFM tip.

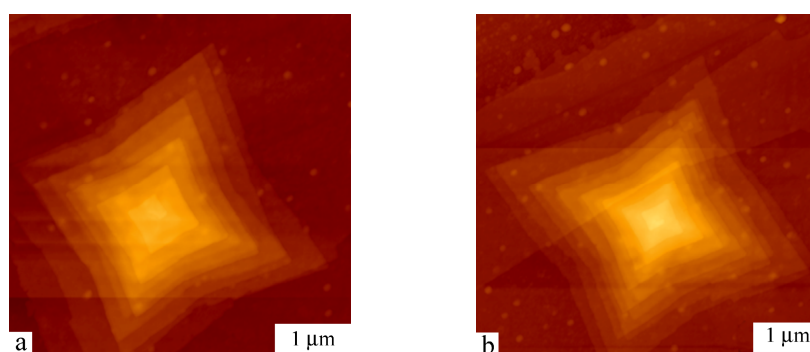


Fig. 1 Typical AFM images of PEO spiral crystal

Figures 2(a) and 2(b) show typical unfolding curves obtained in the normal flat area of PEO spiral crystals in amyl acetate. The unfolding curve, especially in the middle region, shows a force plateau. The force plateau region corresponds to transferring of the polymer fragments from the crystal phase to the outside of the crystal

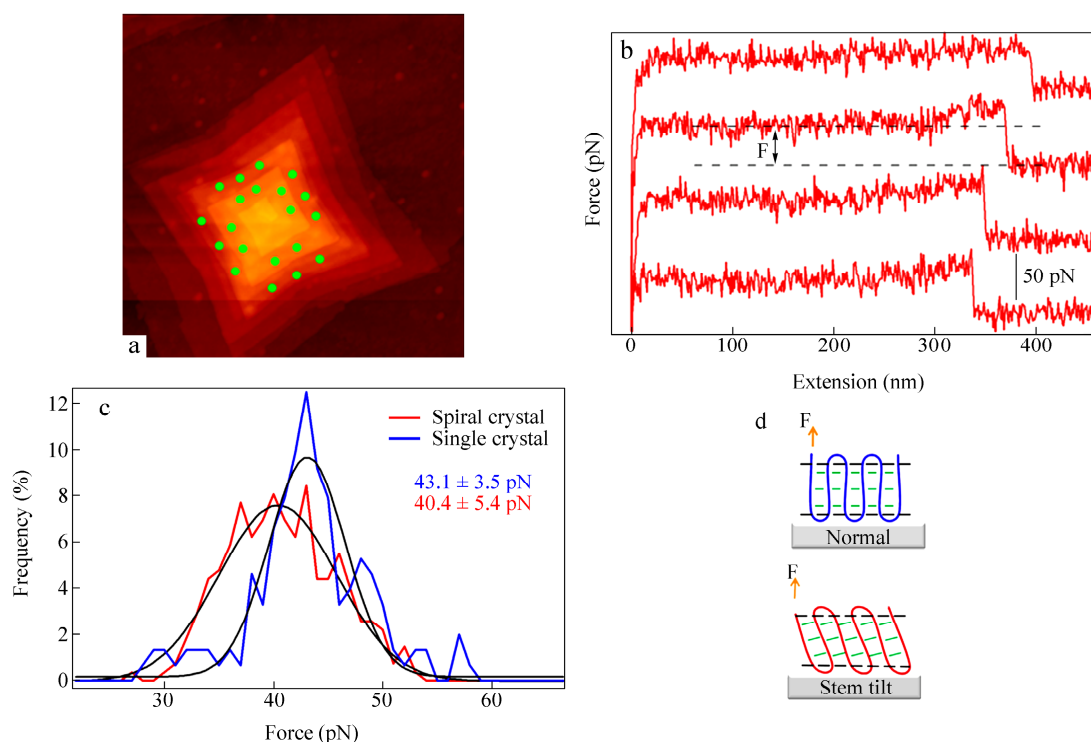


Fig. 2 Detection of the folding mode of a single PEO chain in typical flat area of spiral crystal: (a) Probing positions (colored in green) on the typical area in SMFS experiments; (b) Typical unfolding traces at velocity of 2 $\mu\text{m/s}$; (c) Histogram of unfolding forces in typical area of spiral crystal (colored in red) and normal single crystal (colored in blue) and (d) Schematic drawing for folding modes

(or the amorphous phase), where the free energy change is proportional to the length of solvent-exposed polymer fragment^[12]. Although the shape of the force curves obtained in the flat area of spiral crystal and normal single crystal are identical (indicative of regular folding of PEO chain in both systems), the plateau force in normal single crystal is higher than the force in spiral crystal at the same pulling velocity. Histograms of unfolding forces (Fig. 2c) were plotted from statistical analysis on the average height of the force plateaus (pulling velocity 2 $\mu\text{m/s}$). Gaussian fit gives the most probable forces of ~ 40.4 pN in spiral crystal and ~ 43.1 pN in normal single crystal, respectively. When the polymer chains are unfolded, both unfolding mode (unfolding direction)^[22, 23] and folded height (crystal height or thickness) will lead to different unfolding forces (Fig. 2d). Because the crystallization temperature, which determined the height of crystal, is identical in these two experiments^[24], we anticipate that the difference in the plateau force should mainly be ascribed to the different folding/unfolding modes. From the previous work on DNA pulling, we know that the unfolding force in unzipping loading is much lower than that in uniformly shear loading^[23]. If the stem is tilt relative to the pulling direction (bottom picture in Fig. 2d), the unzipping mode will appear, as a result the unfolding force will decrease. This hypothesis is proved by investigating the single-molecule melting/unfolding force of PEO single crystal immobilized onto the tilted plane. As shown in Fig. 3, the unfolding force in this case is ~ 38 pN, which is smaller than that obtained on normal single crystal immobilized on flat silicon surface (~ 43 pN). Considering the fact that the spiral crystals have been immobilized on flat silicon surfaces and the pulling direction is mostly perpendicular to the crystal surface, we can derive that the stems in the spiral crystal are tilted.

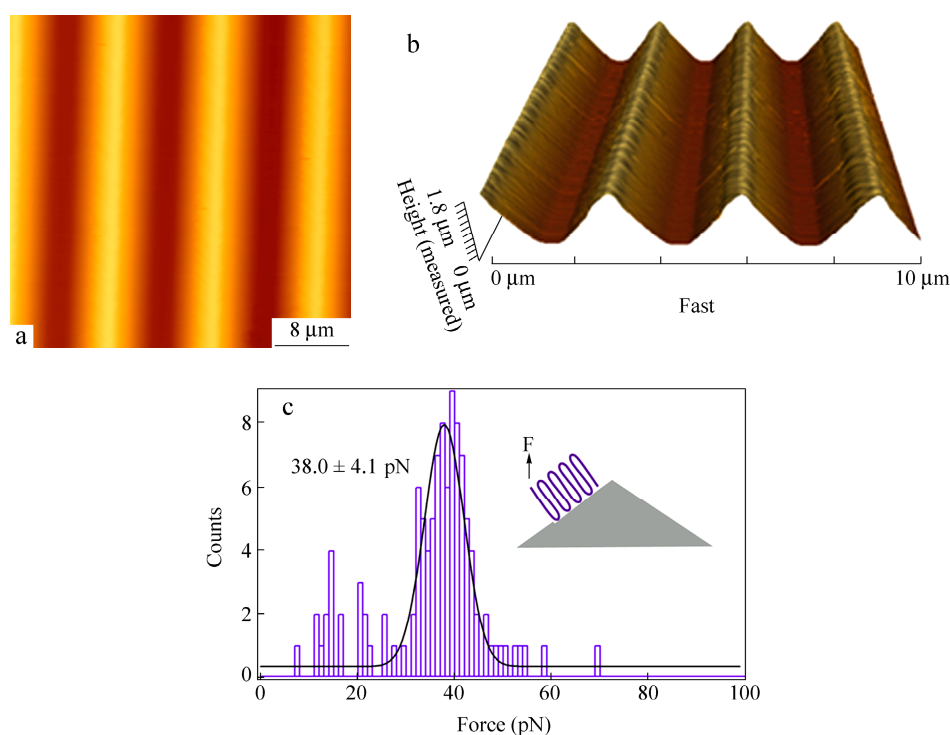


Fig. 3 Unfolding a single PEO chain from single crystal immobilized on an tilted plane: (a) 2-D AFM image of micro-grid; (b) 3-D AFM image of micro-grid and (c) Histogram of unfolding force of single crystal on tilted plane (The inset shows the pulling direction relative to the crystal sample and the surface.)

To further support the previous conclusion, we measured the height (*i.e.*, the thickness) of single-crystal layer, which will decrease when the stem is tilt. The height of single-crystal layer was estimated by indentation experiment, where the AFM tip contacts with the spiral crystal (Fig. 4a) at a constant velocity of 1 $\mu\text{m/s}$. During

the indentation process, a pressure gradient is applied onto the single crystal by the AFM tip. The crystal is elastically deformed, until a sudden drop of tip position appears, which indicates a defined penetration of the tip through the single-crystal layer^[25–27]. At last, the AFM tip touches the solid substrate the force increases linearly again. We call the distance between the first contact of AFM tip with the crystal and the contact of AFM tip with the substrate the breakthrough distance, which is equal to the height of the crystal. Histograms of crystal height (Fig. 4b) are plotted from statistical analysis of the approaching curves. Gaussian fit gives the most probable height of 9.0 nm, which is lower than the height of the PEO single crystal (9.7 nm) prepared at the same temperature, indicating the tilt of PEO stem in spiral crystals.

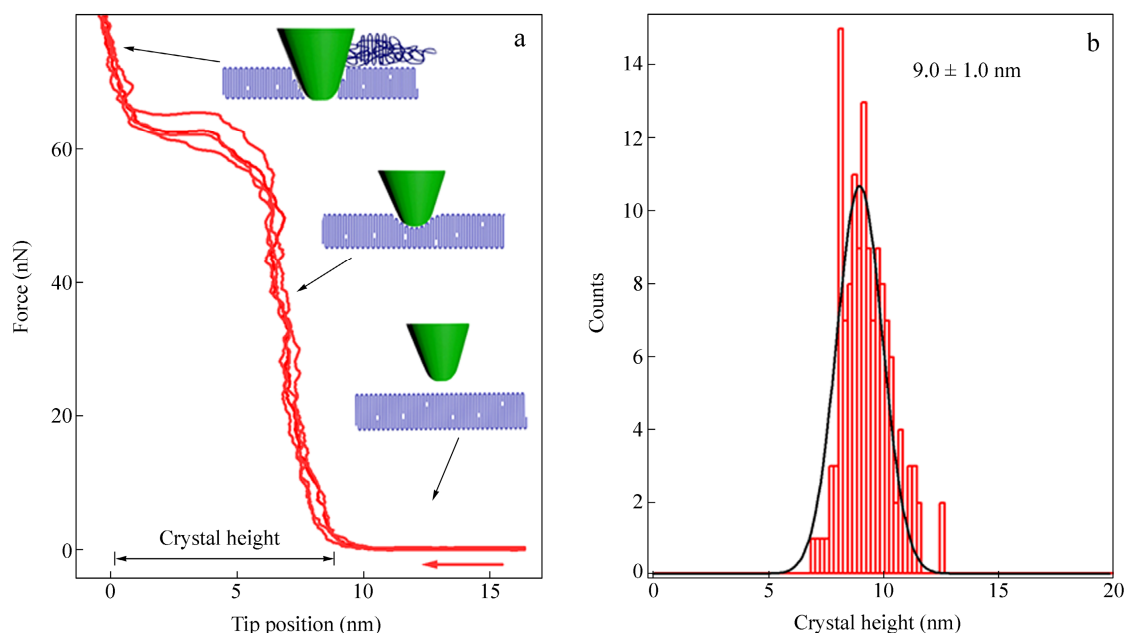


Fig. 4 The determination of crystal height by indentation and penetration experiments: (a) The superimposed indentation curves obtained in flat area on a spiral crystal with an approaching velocity of 1 $\mu\text{m/s}$ (Inset shows a schematic illustration of the indentation and penetration process.) and (b) Histogram of crystal height of the single layer

Folding Mode of a Single PEO Chain in the Area of Screw Dislocation

Apart from the typical flat area of spiral crystal, we have also investigated the folding modes of PEO chains in the area of screw dislocation (Figs. 5a and 5b). Force curves with rising slopes have been obtained in most of the unfolding traces (65% of 500 curves), which were much less (only 14% of 1662 curves) in normal single crystal. The rest 35% of curves show flat plateau with the probable force of 40.5 pN, as obtained in typical area of spiral crystals. To find out the magnitude of tilt more accurately, histograms of average forces in the range of 50–100 nm, 100–150 nm, 150–200 nm and 200–250 nm are built respectively, as shown in Figs. 5(c) and 5(d). The most probable forces increase with the extension, and the probable force was 40.7 pN, 41.5 pN, 44.0 pN and 47.8 pN, respectively. The initial range shows the same force as that in typical flat area, while the force in the range of 200–250 nm is much higher than that in typical area of spiral crystal or single crystal, indicating the gradual increase of crystal height in the region of screw dislocation.

To further prove our hypothesis, the pulling experiment was performed by using a more defined experimental design (nanoparticle positioning)^[21]. The free thiol ends on PEO spiral crystal surface were first labeled by gold nanoparticles *via* thiol-gold chemistry. The gold-coated AFM tip modified with hexanedithiol, leaving the thiol ends on the tip surface, was then positioned over the specific nanoparticle by AFM imaging (the particle marked by the blue circle in Fig. 6a), after which the corresponding PEO chain could be pulled *via* the

manipulation of the attached gold nanoparticle. The absence of the specific nanoparticle in the subsequent AFM imaging (Fig. 6c) indicates that the force signal obtained during the manipulation of nanoparticle (Fig. 6b) does correspond to the pulling of the PEO chain out of its single crystal. Because the density of thiol on crystal surface will increase with the increase of crystal height, the number of PEO chains attached on to a gold nanoparticle will increase accordingly (Fig. 6d). Different from the pulling on typical lamellar single crystal and the typical flat area of spiral crystal, where only 16% and 17.6% of force plateaus are ~ 80 pN, 48.5% of force plateaus obtained in the area of screw dislocation are ~ 80 pN, corresponding to the stretching of double HS-PEO chains attached to a single nanoparticle. This observation proves that the height of the spiral crystal get increased on the area of screw dislocation.

Indentaion and penetration experiments were also used to reveal the change of crystal height in the area of screw dislocation. Figure 7 shows that the height of single-crystal layer gradually increases from the typical area to the screw dislocation area.

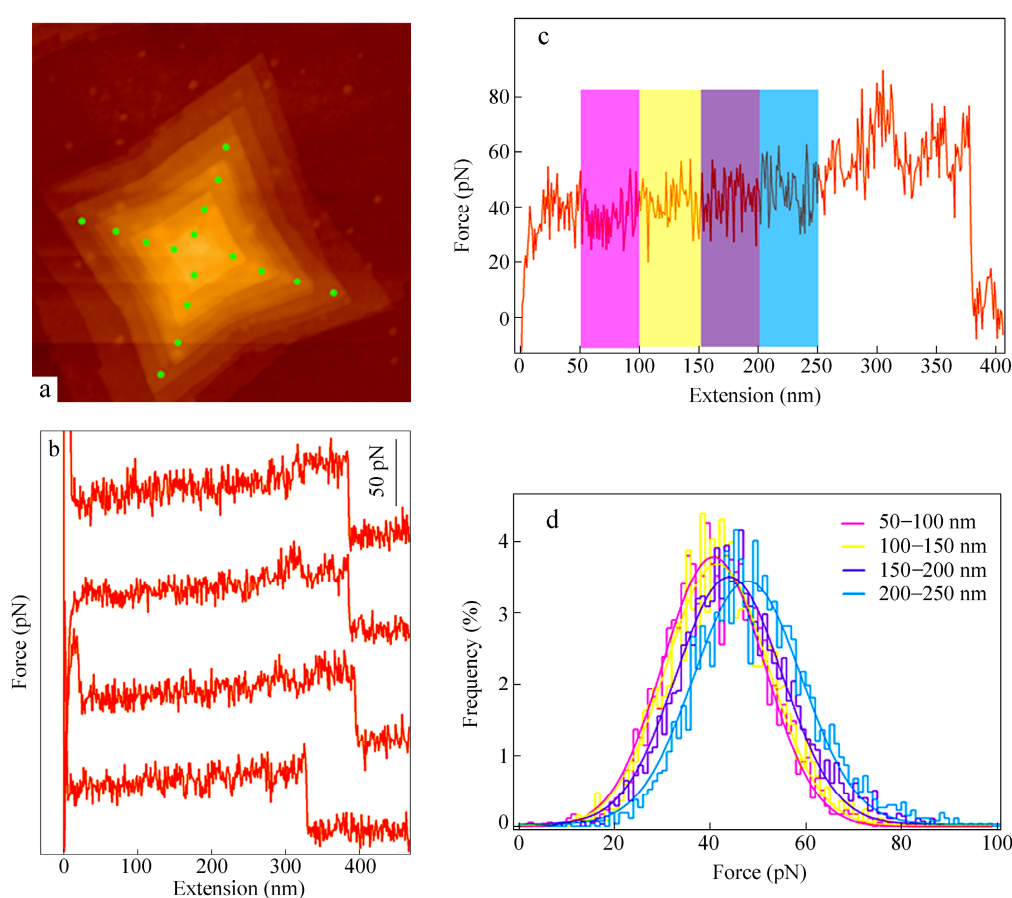


Fig. 5 Folding mode of a single PEO chain on the area of screw dislocation: (a) Probing positions on the area of screw dislocation (colored in green) in SMFS experiments; (b) Typical unfolding traces at velocity of 2 $\mu\text{m/s}$ and (c, d) Histograms of average unfolding forces in the range of 50–100 nm (pink), 100–150 nm (yellow), 150–200 nm (purple) and 200–250 nm (blue)

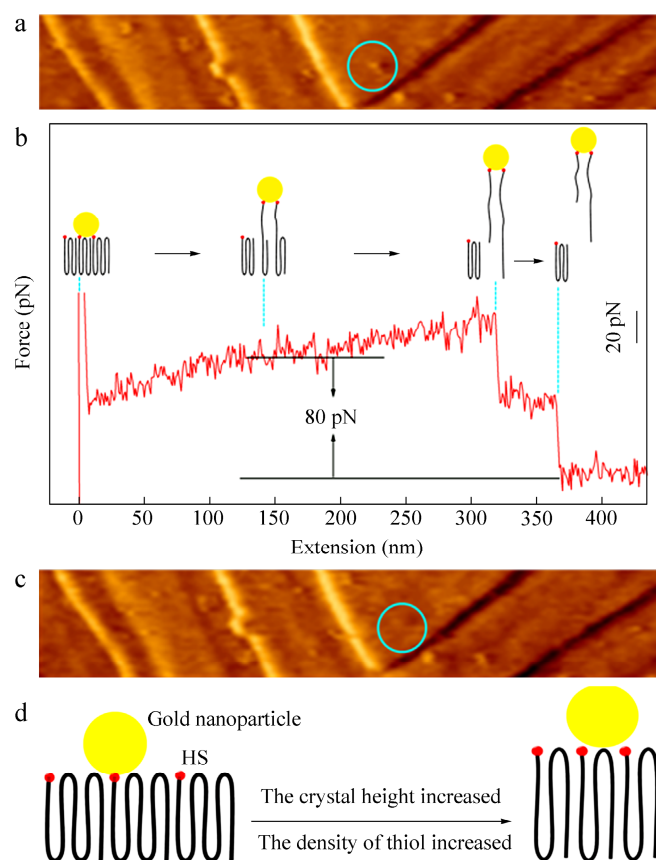


Fig. 6 SMFS experiment on gold-nanoparticle labeled single crystal: (a) Part of the single crystal was imaged first to locate the nanoparticle, the selected nanoparticle marked by the blue circle was then picked up by the AFM tip. (b) The corresponding force-extension signal was recorded. (c) The same part of single crystal was imaged again and the marked nanoparticle has disappeared. (d) Schematic drawing for the relationship between crystal height and density of thiol on crystal surface.

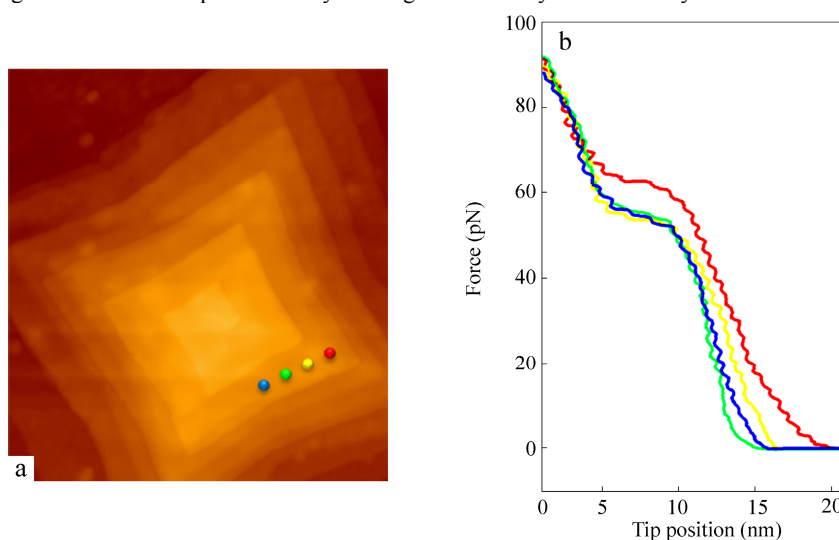


Fig. 7 Crystal height measured by indentation and penetration experiments: (A) Probing positions in the typical area and screw dislocation area of spiral crystal and (B) The superimposed typical curves obtained on spiral crystal with an approaching velocity of $1 \mu\text{m/s}$

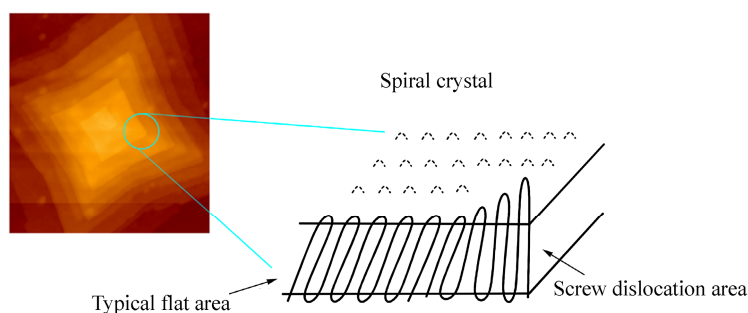


Fig. 8 Schematic diagram for possible folding mode in spiral crystal

CONCLUSIONS

The folding mode of a PEO chain in its spiral crystal has been explored for the first time at single molecule level by a good combination of single-molecule force spectroscopy and AFM imaging. The results demonstrate that the folded stems tilt in the typical area and the crystal height increases in the area of screw dislocation (Fig. 8). The obtained information may be useful for the interpretation of the formation mechanism of spiral crystal. We believe that the method established here can offer a novel strategy to investigate the polymer crystallization and folding mode in other polymer materials at single-molecule level.

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