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Single-Molecule Unidirectional Processive Movement along a Helical Polymer Chain in Non-aqueous Medium

Ken-ichi Shinohara,* Yuu Makida, Takashi Oohashi, and Ryoga Hori

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ABSTRACT: In this work, a molecule "walking" along a single chain of a synthetic helical polymer, which is used as a rail on a substrate in an organic solvent at room temperature, is observed. The walking comprises the unidirectional processive movement of a short-chain molecule along a chiral helical chain in 3 nm steps, driven by Brownian motion and a tapping effect of the atomic force microscopy tip based on a flash ratchet mechanism. Furthermore, the rail consists of a long-chain substituted phenylacetylene polymer with pendant cholesteryl groups, along which the short-chain molecule can walk as a result of van der Waals interactions. The macromolecular motion is videoed using a fast-scanning atomic force microscope, and additionally, this phenomenon is also simulated by all-atom molecular dynamics calculations. On the basis of these results, we propose the principle of a polymer molecular motor. This is the first report of a synthetic walking machine of a chiral helical polymer driven by thermal fluctuation as an artificial life function.
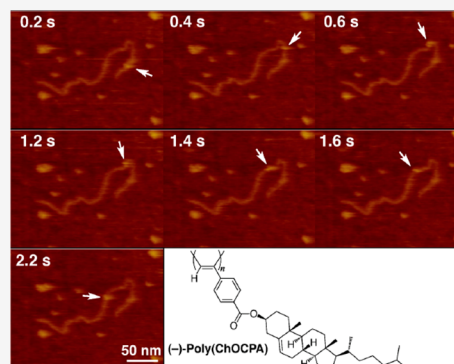
INTRODUCTION

If a single molecule can move unidirectionally to generate force, a molecular motor can be created to transport material and affect morphological change. In living systems, not only are these functions already realized but also highly organized. A synthetic molecular machine with even just some biological functions would require an innovative design concept. The principles by which proteins work in the fields of molecular biology and biophysics have previously been clarified.[1−3] With these concepts applied to the design of synthetic molecules, it should be possible to create molecular machines with characteristics that respond to environmental changes, such as stimuli and loads, that are comparable to biomolecular machines.

Recently reported synthetic molecular machines include catenane,[4] a molecular shuttle called rotaxane,[5] a light-driven molecular rotor that rotates in one direction,[6] and a nanocar,[7] which glides on metal substrates. However, these are small molecules that are limited in terms of the development of more advanced functions, such as substance transport and the generation of force. A molecular machine in which a molecule "walks" along a molecular rail requires the control of intermolecular interactions with the solvent. However, the interaction points in low-molecular-weight molecules are small, and the secondary bonds between molecules dissociate, owing to collisions with the surrounding solvent molecules. Therefore, the molecular walking function disappears. However, it is expected that molecular motors can be produced using polymers capable of dynamic multipoint interactions between molecules.

The motor protein actomyosin, which is found in muscles, is an example of a biomacromolecule. It comprises a complex of the rail protein F-actin and the walking molecule myosin[2] and enables motion. However, artificial synthetic molecules (polymers) have still not been used as molecular walking machines. We speculated whether it would be possible to artificially create a molecular machine like actomyosin. For example, the function of a molecular machine comprising a synthetic polymer could be dictated by molecular design depending upon the method of organic synthesis used, and appropriate stability can be expected.

In the biomolecular motor described above, the actin filament (F-actin) of the rail is formed by the polymerization of spherical G-actin, and the resulting molecule has a chiral helix structure. Myosin walks unidirectionally along F-actin while fluctuating in an aqueous solution, with each step using energy derived from the hydrolysis of adenosine triphosphate (ATP). That is, actomyosin is a molecular motor that is driven by biased Brownian motion.[2]

For a molecular walking machine to function, a rail comprising a periodic structure is required on the surface to function as a scaffold for the walking molecules. Therefore, to fulfill the expected intermolecular interaction, we designed the
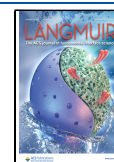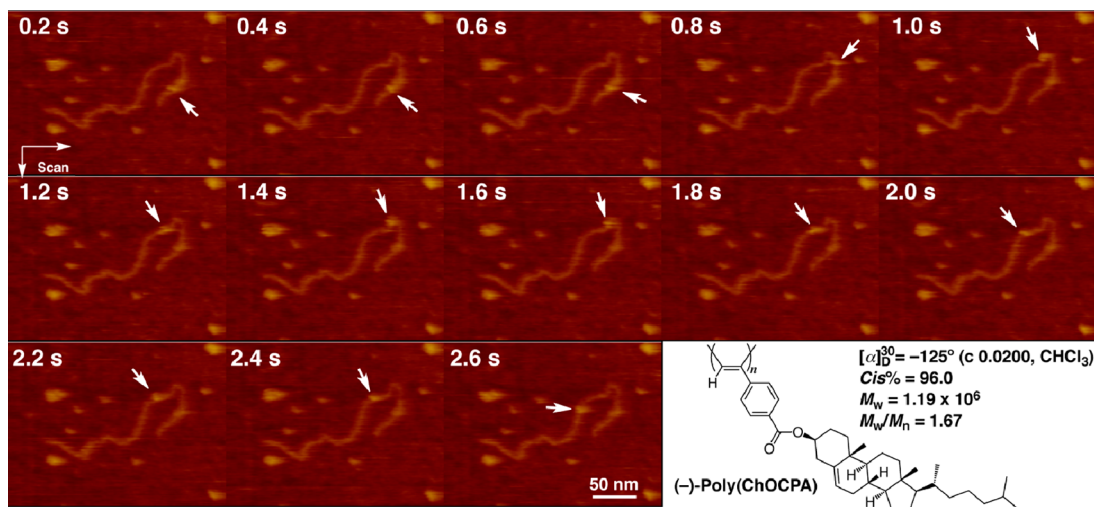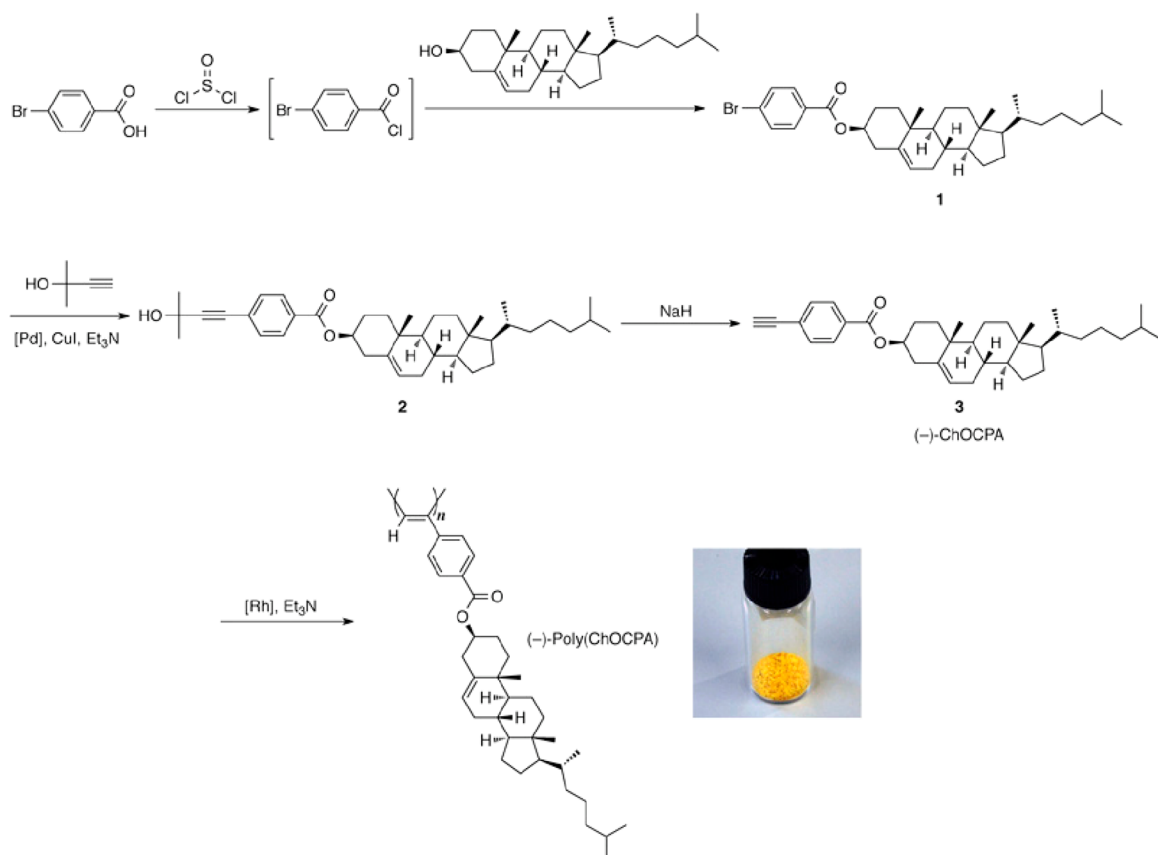
Scheme 1. Synthesis of (−)-Poly(ChOCPA)

Figure 1. Single-molecule imaging of the macromolecular walking function along a chiral helical π-conjugated polymer chain, (−)-poly(ChOCPA), on APS-coated mica under n-octylbenzene at 25 ± 1 °C. White arrows indicate the positions of a walking molecule. *XY*, 250 × 188 nm (320 × 240 pixels); *Z*, 8.40 nm; frame rate, 5.0 frames per second (fps); and X-scan frequency, 1.47 kHz.

main chain polymer with a periodic surface structure comprising a helix and a polymer with pendant cholesteryl groups.[8]

■ EXPERIMENTAL SECTION

To observe a single molecule walking along the polymer chain, we modified the specifications of NVB500 fast-scanning atomic force microscopy (AFM, Olympus, Tokyo, Japan) in dynamic (tapping) mode.[8,9] An ultrasmall cantilever with a low spring constant of approximately 0.1 N/m and a high resonance frequency of over 1

MHz in air was used (AC-10EGS, Olympus, Japan, or USC-F1.2-k0.15, Nano World AG, Switzerland). Fast-scanning AFMs offer outstanding performance for investigating the structural dynamics of single molecules in aqueous solution.[2] However, we modified AFM for use in organic solvents,[10] enabling us to observe the structural dynamics of a single polymer chain (Figure S1 of the Supporting Information).[11,12]

The polymer was dissolved in tetrahydrofuran (THF) to prepare a solution with a concentration of approximately 1×10^{-6} mol/L. The polymer solution was then cast on the surface of a 3-amino-
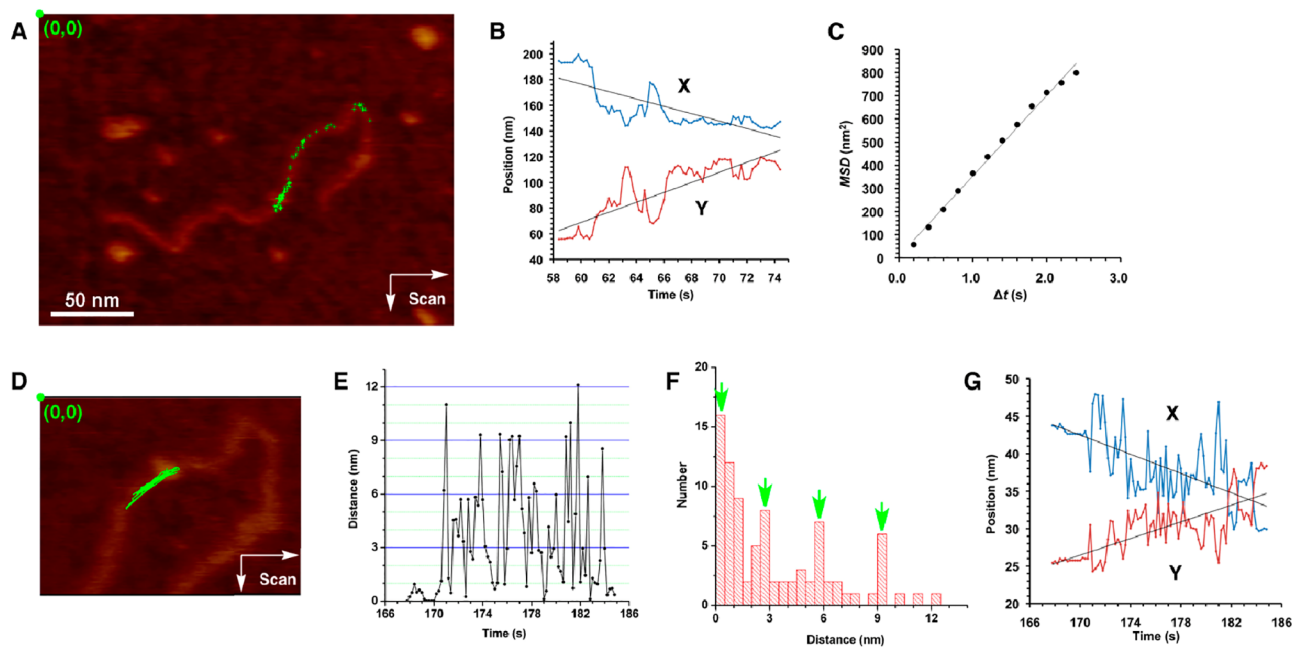
Figure 2. Unidirectional processive movement of a short chain along a chiral helical polymer chain. (A) Short chain walking, observed by AFM video imaging. The X and Y coordinates of the walking molecule were plotted as a green cross in an AFM image. XY, 250×188 nm (320×240 pixels); Z, 8.40 nm; and frame rate, 5.0 fps. The origin (0,0) is in the upper left of the AFM image. (B) Time course of the walking molecule position as X and Y coordinates; the lines were linearly approximated. Unidirectional movement was confirmed. (C) MSD plots based on the trajectory data from panel B; the line linearly approximated the MSD−Δt plots. $D = 86.7$ nm^2/s. (D) Trajectories of the walking molecule and a snapshot from the AFM movie. XY, 90.0×67.5 nm (320×240 pixels); Z, 8.40 nm; and frame rate, 5.0 fps. The origin (0,0) is in the upper left of the AFM image. (E) Time course of the walking distance. (F) Histogram of the distance data from panel E. The green arrows indicate distances of approximately 0, 3, 6, and 9 nm, which correspond to the walking step. (G) Time course of the walking molecule position as X and Y coordinates; the lines were linearly approximated. Unidirectional movement was confirmed.

propyltriethoxysilane (APS)-coated mica substrate[13] to prepare a sample for AFM video imaging.

To determine the dynamics of the walking short chain, the measurement point of the video-imaged short chain was tracked and the mean square displacement (MSD) for a certain time Δt was plotted against Δt.

$$\mathrm{MSD}(\Delta t) = \overline{[\Delta x(\Delta t)]^2 + [\Delta y(\Delta t)]^2}$$

The diffusion coefficient D (nm^2/s) was calculated by dividing the slope of the linearly approximated MSD−Δt plots of the measurement point by 4.

All-atom molecular dynamics (MD) simulations were performed using the Forcite module of the BIOVIA Materials Studio 2019 (Dassault Systèmes BIOVIA, San Diego, CA, U.S.A.) on a supercomputer system (PRIMERGY CX2570 M4, Fujitsu, Tokyo, Japan).

See Figure S2 and Movie S5 of the Supporting Information for more details.

■ RESULTS AND DISCUSSION

A *para*-substituted phenylacetylene polymer with bulky, optically active cholesteryl groups [(−)-poly(ChOCPA)] was synthesized (Scheme 1 and see the Supporting Information for more details). The chiral helical structure was confirmed by circular dichroism (CD) spectroscopy (Figure S3 of the Supporting Information).[14−16] A dilute THF solution of the helical polymer was spin-cast onto a substrate of APS-coated mica, and the single polymer chains were adsorbed and moderately fixed. Subsequently, fast-scanning AFM images were obtained in *n*-octylbenzene at room temperature (Figure 1). As a result, a string-like structure with a length of approximately 300 nm was observed. This size almost agrees

with the value derived from the molecular model, and hence, a single polymer chain was confirmed.

Furthermore, we obtained AFM video images of a short chain (indicated by arrows in Figure 1) walking along a single long chain comprising a chiral helical polymer (Movie S1 of the Supporting Information). This walking short chain is approximately 8 nm long, and its molecular weight is estimated to be several tens of thousands (Figure S4 of the Supporting Information). The walking phenomenon was observed over a long distance (100 nm or more) and for a prolonged time of 4 min or more. The rail consisting of the polymer chain was appropriately fixed on the APS-coated mica substrate, and molecular walking was easily observed using AFM. The instantaneous speed reaches 100 nm/s. In particular, as shown in Figure 1, the observation time was 0.6−1.2 s. The short chain walked along the long chain (the rail) without dissociating, even in the region where the rail polymer chain curved (with a radius of curvature of 10 nm or less). This is an important detail for the function of a molecular walking. The molecular walking was driven by thermal fluctuation. Many walking short-chain molecules have been confirmed, and the results are reproducible (Movies S2 and S3 of the Supporting Information). The diversity of the walking properties is presumed to be due to the variation in the molecular weight of the walking molecule.

In the observation of the unidirectional processive movement of the short-chain molecule along the long polymer chain using AFM video imaging (Figure 2A), it was confirmed on the basis of the analysis of the trajectory of the centroid of the short-chain molecule (Figure 2B). A MSD plot with a linear slope indicates that the molecular motion follows Einstein's
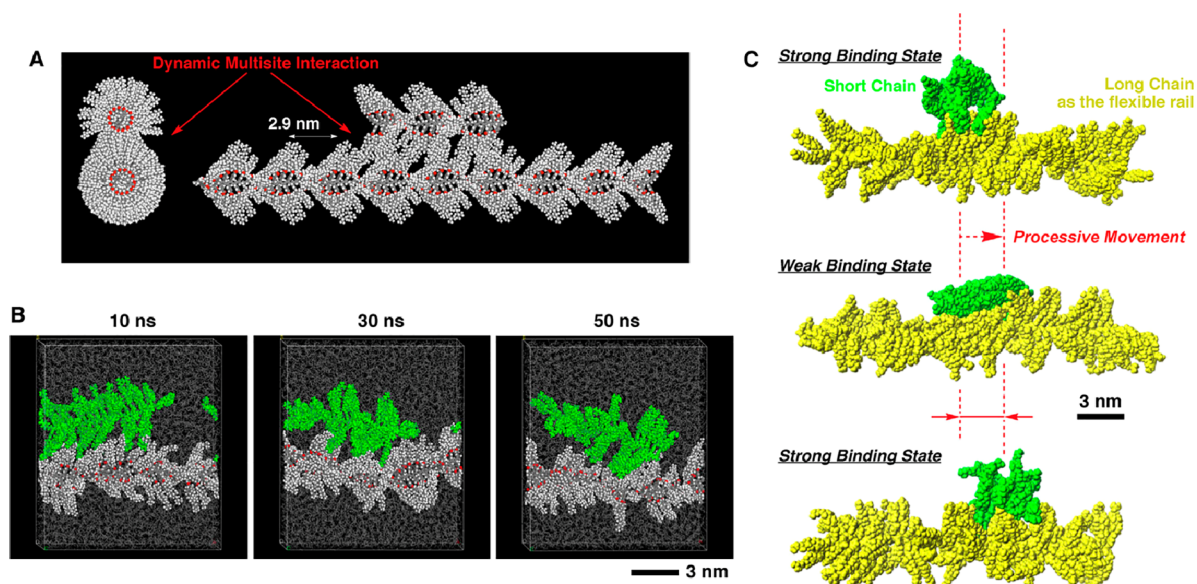
Figure 3. Molecular walking mechanism. (A) Optimized model of an interaction between a short chain (35-mer) and a long chain (120-mer) of (−)-poly(ChOCPA) by MM simulation. (B) Snapshots of all-atom MD by the microcanonical ensemble (*NVE*) after equilibration at 298 K in *n*-octylbenzene (Movie S5 of the Supporting Information). (C) Proposed model of the molecular walking of a short chain (green) along a helical polymer chain (yellow) based on a flash ratchet mechanism.

law of Brownian motion (Figure 2C). The diffusion coefficient was found to be 86.7 nm^2/s. The distribution of the center of gravity of the walking short-chain molecule is shown in Figure 2A. The short chain walked in the direction driven by Brownian motion and the tapping effect of the AFM tip based on a flash ratchet mechanism. At high resolution (0.28 nm/pixel; Figure 2D), 3 nm steps were measured (panels A, E, and F of Figure 2), and unidirectionality was confirmed on the basis of an analysis of the trajectory of the centroid of the short-chain molecule (Figure 2G). As shown in Figure 3A, a pitch of approximately 3 nm was confirmed on the molecular surface of the optimized model by a molecular mechanics (MM) simulation of the helical structure of (−)-poly-(ChOCPA). This periodic structure was considered to function as a "scaffold" for molecular walking. The snapshot structure calculated by all-atom MD simulation of the molecular walking model shown in Figure 3B supports the existence of the dynamic multipoint interaction and molecular-engaged structures. In the all-atom atomic MD calculation, a molecular model, in which the repeating units were bonded at a dihedral angle of 155°, the long chain is infinite, and the short chain comprises a 35-mer (expressed in green), was placed in the MD cell. The solvent molecules (*n*-octylbenzene) were packed at a density of 0.858 g/cm^3. A production run was performed with the microcanonical ensemble (*NVE*) after an equilibration calculation at 298 K from 0 to 60 ns (126 174 atoms). Long-chain movement as a rail has also been confirmed and seems to function as a "moving walkway" carrying short-chain molecules at short distances of a few nm (Movie S5 of the Supporting Information). Along the flexible rail of the helical structure, the short chain moved in steps by dynamic multipoint interactions. Here, a "walking motion by weak bond" and a "walking stop by strong bond" are repeated, and a walking principle that is reminiscent of the crawling locomotion of an inchworm was proposed (Figure 3C). A unidirectionality analysis was also tried (Figure S9 of the Supporting Information).

Processive movement was confirmed. Although this may be due to biased Brownian motion, it remains controversial. This is because even (random) Brownian motion can be unidirectional when observed locally. To settle this discussion, a statistical analysis involving a greater number of observable samples (*n*) is necessary for the future.

Because biased Brownian motion requires an asymmetric potential, the use of chemical energy and macromolecular design for high robustness is considered effective.

Molecular walking in aqueous solutions is well-known in the myosin/actin and kinesin/microtubule systems but has also been demonstrated in non-aqueous solutions. We believe that the present study of synthetic polymer walking will lead to the creation of artificial functional materials, such as artificial muscles driven by thermal fluctuations.[17,18] To create an artificial muscle, it is necessary to assemble the molecules; bundling the polymer chains is expected to make the structure more robust and improve the molecular motor properties.

The research presented here suggests the possibility of discovering other molecular machines that walk in non-aqueous solutions. Unlike biological systems, these synthetical systems are not limited to aqueous solutions. Consequently, molecular machines with various possible chemical structures can be designed.

■ CONCLUSION

The walking comprises the unidirectional processive movement of a short-chain molecule along a chiral helical chain in 3 nm steps, driven by Brownian motion and a tapping effect of the AFM tip based on a flash ratchet mechanism. Furthermore, the rail consists of a long-chain substituted phenylacetylene polymer with pendant cholesteryl groups, along which the short-chain molecule can walk as a result of van der Waals interactions.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.langmuir.2c01704.

AFM head with a fluid cell (Figure S1), snapshot of all-atom MD simulation at 50.0 ns (see Movie S5) and time change of the distance (Figure S2), CD and ultraviolet−visible (UV−vis) spectra of (−)-poly(ChOCPA) (Figure S3), line profile of a short chain on a rail of a long chain (Figure S4), detachment process of the walking molecule from a polymer chain (Figure S5), shapshot of the AFM video (Figure S6), and synthesis of (−)-poly(ChOCPA) (Scheme S1) (PDF)

Single-molecule imaging of the macromolecular walking function along a chiral helical π-conjugated polymer chain, (−)-poly(ChOCPA), on APS-coated mica under n-octylbenzene at 25 ± 1 °C (Movie S1) (MP4)

Unidirectional processive movement of a short chain along a chiral helical polymer chain, with the position of the walking short chain tracked by a green cross in the AFM image (Movie S2) (MP4)

AFM video imaging of molecular walking of several short chains along long chains in n-octylbenzene on APS-coated mica at 25 ± 1 °C (Movie S3) (MP4)

AFM video imaging of an APS-coated mica surface in n-octylbenzene at 25 ± 1 °C as a control experiment (Movie S4) (MP4)

All-atom MD movie in the *NVE* ensemble after equilibration of the supramolecular model of a short chain (35-mer, expressed in green) on a long chain (space-filling model) in n-octylbenzene (line model) at 298 K from 0 to 60 ns as the production run (Movie S5) (MP4)

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Author Contributions

Ken-ichi Shinohara conducted and analyzed the AFM imaging and led the scientific development. Takashi Oohashi and Ryoga Hori prepared and characterized samples. Yuu Makida provided the AFM experiments. Ken-ichi Shinohara wrote the paper.

Notes

The authors declare no competing financial interest.

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