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A Chemo-Mechanical Tweezer for Single Molecular Characterization of Soft Materials

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Abstract

A new Atomic Force Microscopy (AFM)-based chemo-mechanical tweezer has been developed that can measure mechanical properties of individual macromolecules in supramolecular assembly and reveal positions of azide-containing polymers. A key feature of the new technology is the use of an AFM tip densely modified with 4-dibenzocyclooctynols (chemo-mechanical tweezer) that can react with multiple azide containing macromolecules of micelles to give triazole “clicked” compounds, which during retracting phases of AFM imaging are removed from the macromolecular assembly thereby providing a surface topographical image and positions of azide-containing polymers. The force-distance curves gave mechanical properties of removal of individual molecules from a supramolecular assembly. The new chemo-mechanical tweezer will make it possible to characterize molecular details of macromolecular assemblies thereby offering new avenues to tailor properties of such assemblies.

Introduction

The ability to control the composition, structure and functional properties of self-assembled materials is receiving increasing interest due to potential applications such as containers for specific reactions, capture and storage of energy, medical imaging, and drug and gene delivery.[1] [2] While bulk compositions of functionalized self-assembled materials can easily be examined by conventional analytical techniques, it has been difficult to examine chemo-physical properties of individual molecules in an assembly. Here, we report an Atomic Force Microscopy (AFM)-based chemo-mechanical tweezer that can measure mechanical properties of individual macromolecules in supramolecular assembly, reveal positions of azide-containing polymers and provide high-resolution surface topographical images.

AFM, which has a unique capability of imaging nanometer scale structures and measures forces with pN resolution, has been used to image biomolecular structures,[3] unfold single protein molecules,[4] localize single molecule recognition events by breaking molecular

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interactions,[5] break covalent bonds,[6] construct biomolecular assembly structures from the bottom-up[7] and measure interactions between folded polymers.[8]

Here, we report a chemo-mechanical tweezer based on a fundamentally new AFM technology which can measure mechanical properties of individual macromolecules in supramolecular assembly, reveal positions of azide-containing polymers and provide high resolution surface topographical images. In this approach, a magnetically driven AFM tip is modified with a reagent that can react with a functional group present at the terminus of a macromolecule (Figure 1). A reaction between the two functional groups was expected to take place during the contact phase of AFM imaging. Although this event will result in the formation of a covalent bond, the retraction phase will not result in breakage of such a linkage because non-covalent interactions that hold together the macromolecules in the self-assembled material are much weaker and therefore this step will result in the extraction of a macromolecule. The resulting force curves will provide mechanical properties of removal of individual macromolecules and furthermore, by delineating perturbations in sinusoidal oscillation of the cantilever, as in picoTRAC imaging,[5] surface topography and recognition events will be resolved in a temporal and spatial manner. In this approach, a functional group of a tip can only be used once, and hence a critical issue was to modify AFM tips with a sufficient number of reactive groups for making a meaningful number of measurements. Another key issue was the selection of functional groups that react fast, are compatible with a complex environment, yet sufficiently stable for tip and macromolecule modification. The advent of click chemistry, in particular the copper (I)-catalyzed azide-alkyne cycloaddition and the strain promoted alkyne-azides cycloaddition which both give stable triazoles, provide such reaction pairs.[9] For the purpose of this study, we employed azide-containing amphiphilic block copolymers (**1**)[10] and dibenzocyclooctynols (**5** and **6**)[11] for tip modification.

Results and Discussion

Compound and Micelle Preparation

To implement the new technology, a range of multifunctional organomicelles (**A, D**–**F**, Table 1) was prepared by the addition of various mixtures of amphiphilic block copolymers (**1**–**4**, Scheme 1) to water.[1b] Thus, copolymer **1** will assemble into organomicelles carrying azido functions at its surface. The additional use of block copolymer **2**, which carries a thioctic moiety[12] at the polar terminus, was expected to offer an opportunity to attach the resulting particles to a gold surface. Unfunctionalized block copolymer **3** allowed controlling the surface density of the functional groups. Copolymer **4** has a similar structure as **3**, however, it contains an azido function at the apolar poly(ε-carprolactone), which was expected to reside at the internal environment of resulting micelles. Compounds **5** and **6**, which are composed of a 4-dibenzocyclooctynol linked through an oligoethylene glycol linker to a bromoacetyl group, were employed for AFM tip modification. These two compounds differ in the length of the oligoethylene glycol linker, which was expected to influence the number of recognition events that can be made by tips modified with these compounds. Finally compound **7**, which contains a thioctic acid at the apolar PCL terminus for attachment to a gold-surface and an azide at the polar PEG terminus for reaction with dibenzylcyclooctynol of the tip was prepared, to establish the force required for breaking a covalent bond of the linker.

Azido polyethylene glycol (**9**), which is a precursor for the preparation of block polymer **1**, was prepared by monotosylation of polyethylene glycol **8** (n ≈ 45) with tosyl chloride in pyridine followed by displacement with sodium azide in DMF. Polymerization of azido- (**7**) and commercially available methoxy polyethylene glycol (n ≈ 45) with caprolactone in the presence of tin(II) 2-ethylhexanoate (SnOct) at 130°C for 24 h gave **1** and **3**,

respectively[10a] (m ≈ 26, M_n = 5000, PDI ≈ 1.4). Thioctic acid modified copolymer **2** was prepared from **1** by reduction of the azido group by catalytic hydrogenation of Pd/C followed by acylation of the resulting compound **10** with the *N*-hydroxysuccinimate ester of thioctic acid in the presence of triethylamine. Compound **4** was obtained by acylation of the hydroxyl of the PCL terminus of **3** with 6-azidohexanoic acid in the presence of DCC and DMAP in dichloromethane.

Compound **5** was easily prepared by a three-step procedure involving reduction of the azido moiety of **9** (n ≈ 23) to give **12**, which was then acylated with **14** followed by activation of the hydroxyl of the resulting compound **15** by reaction with bromoacetyl bromide. Compound **6** was prepared by a similar procedure employing an appropriately modified azido oligoethylene glycol. A range of micelles was prepared by addition of nanopure water to a mixture of block copolymers in THF (Table 1) followed by dialysis. Organomicelles **A** are composed of block copolymers **1**, **2** and **3**, and hence can be immobilized on a gold surface and are expected to display surface azido functions. Micelles **B** and **C** have a similar composition as **A** but are loaded with a cytotoxic drug and a fluorescent dye, respectively. These preparations are expected to be attractive for drug delivery and localization studies, respectively and furthermore, may have different stabilities due to loading. Micelles **D** are composed of block copolymers **2** and **3** and therefore can be immobilized on a gold surface but do not express azido moieties. Micelle **E** will present thioctic acid moiety at the surface, however, the azido groups will be buried in interior of the micelles. Finally, preparation of **F**, which is composed of block copolymers **1** and **3**, does not contain thioc acids and therefore cannot adhere to a gold surface.

AFM imaging

First, the size, shape and surface morphology of the various multifunctional micelles in their native environment was visualized by Agilent MAC mode AFM scanning using an unfunctionalized tip. The organomicelles were attached to an Au(1,1,1) surface *via* thiol-gold linkages (Figure 1) and unbound material was removed by washing with water. As can be seen in Figure 2, the organomicelles **A** are uniformly distributed and have diameters in the range of 20–50 nm, which agrees with the dynamic light scattering (DLS) measurements (SI-2). The topographic, amplitude and phase images show fine structural details of the micelle surface, and in particular the phase and amplitude images reveal molecular details. As expected, organomicelles **B**, **C**, **D** and **E** gave similar high-resolution images (Figure SI-2). Micelles **F**, which do not contain surface thiol moieties, had a similar size as the other micelles, but did not show molecular details highlighting the importance of immobilization for high-resolution imaging. Furthermore, time-lapse measurements showed movement of these micelles over the surface (Figure SI-2).

To detect surface azido moieties, AFM tips were modified with a dense layer of 4-dibenzocyclooctynol derivative **5** (Figure 3). Due to the relatively long polyethylene glycol linker (~9 nm) of **5**, it was expected that 4-dibenzylcyclooctynols attached to the top and cone of the tip would be available for reaction and it was estimated that ~1000 molecules would be available for reaction (SI-4). High-density modification was achieved by exposing tips to *pure* (3-mercaptopropyl)-triethoxysilane followed by the addition of a *large excess* of **5**. Fluorescence images of tips reacted with AlexaFluor®488-azide- and rhodamine-B-loaded micelles **C** confirmed proper tip modification (SI-3, Figure SI-3). The conditions used here are very different from tip modification for recognition AFM, which uses highly diluted solutions of silane and probe molecule.[5]

The topographic image of micelles **A** (bright spots, Figure 2d, top) obtained by using an unmodified tip clearly demonstrated the presence of micelles. As expected, the recognition image did not reveal reaction events (Figure 2d, bottom). However, imaging micelles **A** with

a 4-dibenzocyclooctynol-modified tip revealed specific recognition events as represented by the dark spots in the recognition image (Figure 2e, bottom). As expected, no recognition was observed when a similar experiment was performed with organomicelles **D** and **E**, which do not contain azido moieties or have azido groups at the interior of the micelle, respectively (Figure SI-7–1). A mixture of organomicelles **A** and **D** was investigated and as expected, the two types of micelles were indistinguishable in the topographic image (Figure 2f, top). However, the recognition image (Figure 2f, bottom) clearly demonstrated recognition in some but not all of the micelles. These results unambiguously demonstrate that recognition events only occur when the 4-dibenzocyclooctynols attached to the tip chemically reacts with azide moiety at the surface of organomicelles.

To establish the number of recognition events that can be measured by a single tip, *in-situ* time-laps recognition imaging of micelles **A** was performed using a tip functionalized with **5** (~9 nm spacer) and **6** (~2 nm spacer) and 300–500 and 50–60 recognition events could be realized, respectively (Figure SI-7–2). These observations support the notion that the use of a longer spacer makes a larger number of recognition molecules available for reaction.

The probability of removal of a single polymer increased from approximately 14% to 30% when the retention time was increased from 0 to 600 ms (Figure 4a,b). Furthermore, the probability of observing force curves resembling the removal of two or three polymers increased with longer retention times. The high probability of reaction can be rationalized by the fact that during the contact phase, high-density surfaces of azides and 4-dibenzocyclooctynols are mechanically brought together resulting in a very high local concentration of reactants. Estimated local concentrations provide a reaction rate that is comparable with the experimental results (SI-5).

The recognition images revealed the presence of azide moieties at the surface of the micelles; but could not distinguish between micelles **A** and **B** or **C,** which are composed of the same block copolymers but differ in the absence or presence of a load. Hydrophobic forces are an important determinant of the assembly of organomicelles in water and therefore,[13] it was expected that loading would influence the force required for removal of a block copolymer molecule from a micelle. In particular, the `pull out' forces were significantly larger for micelles **B** (65.0±3.3 pN) and **C** (67.8±0.7 pN), which were loaded with doxorubicin and rhodamine, respectively compared to unloaded micelles **A** (49.8±1.9 pN) at a force-loading rate of 15 nN/s (Figure SI-8–1). The stretching distances were also significantly longer for **B** (13.5±0.4 nm) and **C** (9.3±0.3 nm) compared to **A** (5.6±0.2 nm) (Figure SI-8–1). The critical micelle concentration for **A** was higher than that for **B** and **C** (SI-2), supporting differences in stability of the two preparations. The increased stability of the micelles is probably due to hydrophobic interactions between the apolar loading material and the PCL component of the micelles. The difference in breaking forces and stretching distance for **B** and **C** are probably due to differences in loading content and molecular characteristics of the load.

To further support that the above measured forces are due to removal of polymeric molecules from a micellar structure, a monolayer of compound **7** (Figure 3) on Au(1,1,1) surface was formed and examined by AFM imaging using a dibenzylcyclooctynol modified tip. Compound **7** contains a thioctic acid moiety at the apolar PCL terminus for attachment to a gold-surface and an azide at the polar PEG terminus for reaction with dibenzylcyclooctynol of the tip. Removal of **7** from the surface by reaction with **5** of the tip can only be accomplished by breaking a covalent bond. A rupture force of 338.5±6.3 pN at a force-loading rate of 2000 nN/s was measured (from a 1000 distance curves, SI-6), which is ~10-fold larger than the forces required for removal of a polymer from a micelle and is in

agreement with previously reported rupture force of thiol-gold linkages.[14] The other covalent bonds of system require even larger forces (nN range) for breakage.[6]

Over the course of an experiment, polymeric molecules will cover the tip, which may affect breaking forces. However, it was found that the most probable forces for micelles **A** recorded at three subsequent eight minutes intervals were 48.8±3.7 pN, 44.0±1.3 pN and 44.4±1.0 pN, whereby the difference in these values is within the error of the measurement (Figure SI-8–2). Click chemistry is transforming many areas of chemistry and material and life sciences.[9] It has for example been used in conjunction with AFM to pattern surfaces. In one application, an azide is delivered by dip-pen nanolithography to a surface modified with silicon surface modified with alkyne in the presence of a Cu(I) catalyst.[15] In another approach, an AFM tip was modified by a Cu(I) catalyst and employed for spatially controlled reactions of alkynes with immobilized acides.[8b]

Here, we demonstrate that distributions, surface topography and mechanical properties of individual molecules of a complex assembly can be examined by modifying an AFM tip and components of a supramolecular assembly with a pair of click reagents. The fact that the azido function is small, inert and can be selectively installed by chemical or biological approaches[9c] makes the new methodology versatile. The approach has, however, as a potential drawback that a limited number of measurements can be made due to the fact that the clickable groups modified on the AFM tip are being used during the scanning process. It has, however, been found that more that between 500–1000 recognition events can be made when the click reagent is linked to the AFM tip by a sufficiently long linker. This number is sufficiently large to characterize a self-assembled material and furthermore, tip modification is a relatively straightforward and thus it is possible to examine multiple materials.

It is to be expected that by comparing macroscopic properties with behaviour of individual molecules, fundamental properties of complex synthetic and biological self-assembled materials can be uncovered.

Experimental Section

Synthesis of copolymer N_3-PEG-*b*-PCL (1)

N_3-PEO-*b*-PCL was synthesized by one-pot cation ring opening polymerization at 130°C under a stream of argon as previously reported for the preparation of PEO-*b*-PCL with some modifications. Briefly, ε-caprolactone monomer (3.5 mL, 31.6 mmol) was added into a flask containing of N_3-PEG-OH (**9**, see SI-1 for preparation) (2.5 g, 1.235 mmol) and the resulting mixture was placed under a nitrogen atmosphere and then a drop of SnOct was added. The mixture was cooled by placing in bath filled with liquid-nitrogen and then evacuated, sealed off and kept at 130°C for 24 h. The resulting polymer was dissolved in THF (20 mL), precipitated by the addition of cold hexane (1000 ml), collected by filtration and then dried in vacuum at room temperature to give the product as a white solid (5.5 g, ~91%). The degree of the polymerization of the PCL and the polydispersity of the polymers were determined by SEC (Figure SI-1–2). The degree of polymerization of the PCL was also measured by ^{1}H NMR (Figure SI-1–3) relative to the degree of polymerization of the PEO. ^{1}H NMR ($CDCl_3$, 300 MHz) δ= 4.10−4.02 (52H, m; $CH_2CH_2CH_2O$), 3.80−3.58 (178H, m; CH_2O), 3.36 (2H, m; CH_2N_3), 2.26−2.20 (52H, m; $CH_2C{=}O$), 1.65−1.55 (104H, m; CH_2), 1.30−1.22 (52H, m; CH_2). M_n (SEC): 6070 (polydispersity index (PDI) = 1.46). FT-IR spectrum of **1** is shown in Figure SI-1–4.

Synthesis of copolymer TA-PEG-*b*-PCL (2)

A suspension of copolymer **1** (500 mg) and 10% Pd/C (100 mg) in ethanol (30 mL) was stirred under an atmosphere of H_2 at room temperature for 15 h. The catalyst was removed

by filtration and the filtrate was concentrated in vacuo to give **10**. A solution of **10** (450 mg) and triethylamine (0.1 g, 1.0 mmol) in CH_2Cl_2 (20 mL) was placed under an atmosphere of Ar and kept cooled (4°C). Then a solution of TA-NHS (0.3 g, 1.0 mmol, see SI-1 for preparation) in CH_2Cl_2 (5 mL) was added dropwise to the solution over a period of 1 h. The reaction mixture was warmed to room temperature and stirring was continued for 18 h.

The mixture was concentrated *in vacuo* and the residue purified by Sephadex LH-20 size-exclusion chromatography (CH_2Cl_2/CH_3OH, 1/1 v/v). Concentration of the appropriate fractions afforded **2** as a white solid (418 mg, 81%).^{1}H NMR ($CDCl_3$, 300 MHz) δ= 4.10−4.02 (52H, m; $CH_2CH_2CH_2O$), 3.80− 3.58 (180H, m; CH_2O), 2.38−2.41 (3H, m; S–CH_2–CH_2–CH-S), 2.26−2.20 (52H, m; CH_2C=O), 1.83 (2H, m; S–CH_2–CH_2–CH–S), 1.65−1.55 (104H, m; CH_2), 1.46 (4H, m; CH_2–CH_2–CH_2–CH_2–CO), 1.30−1.22 (52H, m; CH_2). FT-IR spectrum of **2** is shown in Figure SI-1–5.

Synthesis of copolymer PEG-*b*-PCL (3)

A one-pot cation ring opening polymerization at 130°C under a stream of argon was adapted from a previously reported preparation of PEO-*b*-PCL[10a] with some modifications. Briefly, ε-caprolactone monomer (3.5 mL, 31.6 mmol) and MeO-PEG-OH (**11**) (2.5 g, 1.25 mmol) were placed under a nitrogen atmosphere and then a drop of SnOct was added. The mixture was cooled by placing in bath filled with liquid-nitrogen and then evacuated, sealed off and kept at 130°C for 24 h. The resulting polymer was dissolved in THF (20 mL), precipitation by the addition of cold hexane (1000 mL), collected by filtration and then dried *in vacuo* at room temperature to give the product as a white solid (5.4 g, ~90%). The degree of the polymerization of the PCL and the polydispersity of the polymers were determined by SEC (Figure SI-1–6). The degree of polymerization of the PCL was also measured by ^{1}H NMR relative to the degree of polymerization of the PEO. ^{1}H NMR ($CDCl_3$, 300 MHz) δ= 4.10−4.02 (52H, m; $CH_2CH_2CH_2O$), 3.80−3.58 (180H, m; CH_2O), 3.38 (3H, s; CH_3O), 2.26−2.20 (52H, m; CH_2C=O), 1.65−1.55 (104H, m; CH_2), 1.30−1.22 (52H, m; CH_2). M_n (SEC): 6010 (polydispersity index (PDI) = 1.53).). The FT-IR spectrum of **3** is shown in Figure SI-1–7.

Synthesis of copolymer PEG-*b*-PCL-N_3 (4)

A solution of polymer **3** (0.5 g, 0.1 mmol) and 6-azido-hexanoic acid[16] (0.157 g, 1.0 mmol) in CH_2Cl_2 (30 ml) was added to *N*,*N*′-dicyclohexylcarbodiimide (0.206 g, 1.0 mmol) and 4-dimethylaminopyridine (catalytic amount). The mixture was stirred for 18 h. The white precipitate was filtered off and washed with a minimal amount of CH_2Cl_2. The filtrate was concentrated under reduced pressure and the residue was purified by Sephadex LH-20 size-exclusion chromatography (CH_2Cl_2/CH_3OH, 1/1 v/v) to give **4** as a white solid (432 mg, 84%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ= 4.10−4.02 (52H, m; $CH_2CH_2CH_2O$), 3.80−3.58 (180H, m; CH_2O), 2.26−2.20 (54H, m; CH_2C=O), 1.65−1.55 (108H, m; CH_2), 1.30−1.22 (56H, m; CH_2). FT-IR spectrum is shown in Figure SI-1–8.

Synthesis of 5

A solution of **15** (100 mg, 0.02 mmol, aee SI-1 for synthesis) in CH_2Cl_2 (15 mL) was placed under an atmosphere of Argon and then bromoacetyl bromide (40 mg, 0.2 mmol) and NEt_3 (30 mg, 0.3 mmol) were added. After stirring the reaction mixture for 18 h at ambient temperature, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 30/1, v/v) to afford **5** (66 mg, 67%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ= 7.33−6.96 (8H, m; aromatics), 5.42 (1H, m; $CHOC$=O), 4.23 (2H, m; CH_2Br), 3.67 (2H, m; CH_2OH), 3.65−3.57 (180H, m; CH_2O), 3.33 (2H, m; CH_2NH); 3.07 (1H, m; ArCH_2), 2.89 (1H, m; ArCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ= 166.5, 154.7, 152.4, 151.2, 129.7, 129.2, 128.0, 127.2, 126.9, 126.5, 126.0,

125.1, 124.9, 122.8, 122.2, 111.8 (alkyne), 108.9 (alkyne), 75.6, 71.6, 70.4, 69.5, 69.4, 69.2, 69.1, 69.0, 68.6, 66.7, 60.6, 45.1, 39.8, 28.6.

General procedure for the preparation organomicelles A, D, E and F

A mixture block copolymers (10 mg) in THF (1.0 mL) was slowly added to water (15 mL) under sonication. The final mixture was opened to air overnight, allowing slow evaporation of THF and formation of micelles, then dialyzed against 2 L of nanopure water (pre-swollen semi-permeable membrane: cutoff 12,000 – 14,000 Da) for 4 h, the water was replaced every h.

General procedure for the preparation of loaded organomicelles (B and C)

Doxorubicin or rhodamine B (1 mg) was added to a solution of block copolymer **1**, **2** and **3** (10 mg) in THF (1.0 mL). The mixture was slowly added to water (15 mL) under sonication. The final mixture was opened to air overnight, allowing slow evaporation of THF and formation of micelles, and then dialyzed against 2 L of nanopure water (pre-swollen semi-permeable membrane: cutoff 12,000 – 14,000 Da) for 4 h, the water was replaced every h. The micelle solution was passed through a syringe filter (pore size 0.45 μm; Millipore, Billerica, MA) to remove doxorubicin or rhodamine B aggregates.

Determination of doxorubicin or rhodamine B loading content of micelles B and C

The loading content of micelles **B** and **C** was defined as the weight percentage of doxorubicin or rhodamine B in the micelle and quantified by fluorescence intensity measured on BMG Labtech POLAR star optima. First, the micelle **B** or **C** solutions were frozen and lyophilized to give a solid sample. The dried samples were redissolved in a mixture of chloroform and DMSO (1:1, v/v) for fluorescence measurements. The sample data are compared with standard curve of fluorescence intensities *vs.* various concentration solutions of doxorubicin or rhodamine B.

AFM sample preparation

A fresh thermal evaporated gold surface was annealed by hydrogen flame, which was immediately covered with a nanoparticle solution (1 mg/mL) for 2 h at 4 °C. The surface was rinsed three times with 18 MΩ DI water and then examined by AFM. For recognition experiment, a ten-fold diluted solution of nano-particles was employed.

AFM experimental procedures

An Agilent 5500 AFM system equipped with an inverted light microscope (ILM) system (Agilent, Chandler, AZ) was employed for scanning an area of 10 μm^2. Silicon cantilever tips with a nominal spring constant of about 0.1 N/m were used throughout the experiments. All images were collected in water using recognition imaging module based on Agilent magnetic AC (MAC) mode AFM with a magnetically coated lever. For the force microscopy study, the AFM cantilevers were stretched under several different pulling rates, range from 300–4000 nm/s. For each pulling rate, 1000 pulling trajectories (*i.e.* force-distance curves) were recorded at room temperature. The statistical histograms of force-distance curves were obtained from a subset of the pulling traces that represented the successful binding events. In the statistical study, the apparent loading rate was applied to attain the loading rate dependence of force and stretching distance.

High resolution images

Ultrahigh resolution images were obtained by AFM at Top Magnetic AC (TOPMAC) mode for micelles **B** (Figure SI-2a), micelles **C** (Figure SI-2b), micelles **D** (Figure SI-2c) and micelles **E** (Figure SI-2d). The micelles were uniformly distributed throughout the gold

surface and have diameters ranging from 20–50 nm. Notably, micelles **F**, which do not have surface thiol moieties for immobilization, had a similar size range as the other micelles; however, the images did not show molecular details of the micellar surface (Figure SI-2e). This observation highlights the importance of immobilizing micelles for achieving high resolution images. Furthermore, the micelles **F** appeared to move over the surface as shown in the topographic images re-measured after lapse periods of 8 min (Figure SI-2e–h). Tip modification is depicted in Figure SI-3–1a.

AFM tip modification

Tips were cleaned by UV for 30 min and then coated with a magnetic film by the e-beam deposition. The tips were immediately placed in a small container in a glass desiccator filled with argon. Next, (3-mercaptopropyl)triethoxysilane (20 μL) and *N*,*N*-diisopropylethylamine (10 μL) were added to the small containers; and then the desiccator was placed under a reduced pressure at 1 torr for 60 min.[17] The organo-silicon coated tips were washed with hexane for 15 min using sonication and then immersed in the solution of compound **5** (8.8 mg, 7.15 μmol) in DMF (400 μL) and triethylamine (5 μL) for 5 h. Finally, the tips were rinsed several times with water and kept in pure water at 4 °C.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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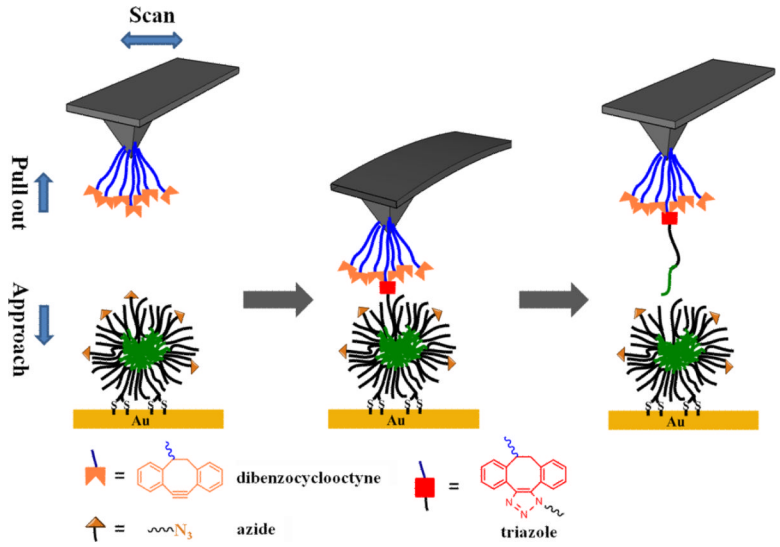
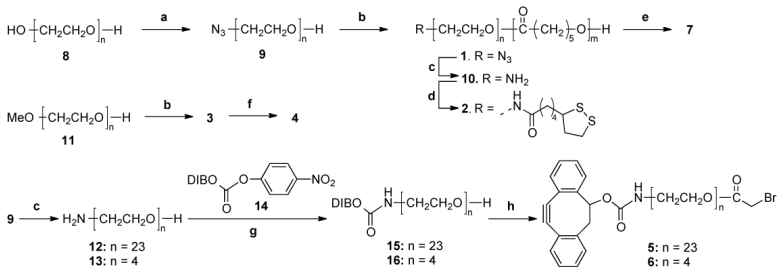


Figure 1.

Schematic illustration of AFM-based method for probing functional groups of self-assembled materials. Multifunctional organomicelles modified with surface azido and thioic acid moieties are immobilized on Au(111) surface *via* thiol-Au bonding. AFM tips are modified with a dense layer of dibenzocyclooctynols. During the contacting phase, a reaction between an azide of a micelle with a dibenzocyclooctynol of the tip can occur resulting in the formation of a triazole. The occurrence of such a reaction can be detected in the recognition image by removal of the “clicked”-polymer from the supramolecular assembly.



Scheme 1.

Chemical synthesis of compounds **1–7**. Reagents and conditions: a) TsCl, pyridine then NaN_3, DMF, 80°C; b) ε-caprolactone, SnOct, 130°C; c) Pd/C, H2, EtOH; d) DL-thioctic acid-NHS, TEA, CH_2Cl_2; e) DL-thioctic acid, DCC, DMAP, CH_2Cl_2; f) $N_3(CH_2)_5CO_2H$, DCC, DMAP, CH_2Cl_2; g) Et_3N, CH_2Cl_2; h) bromoacetyl bromide, Et_3N, CH_2Cl_2.

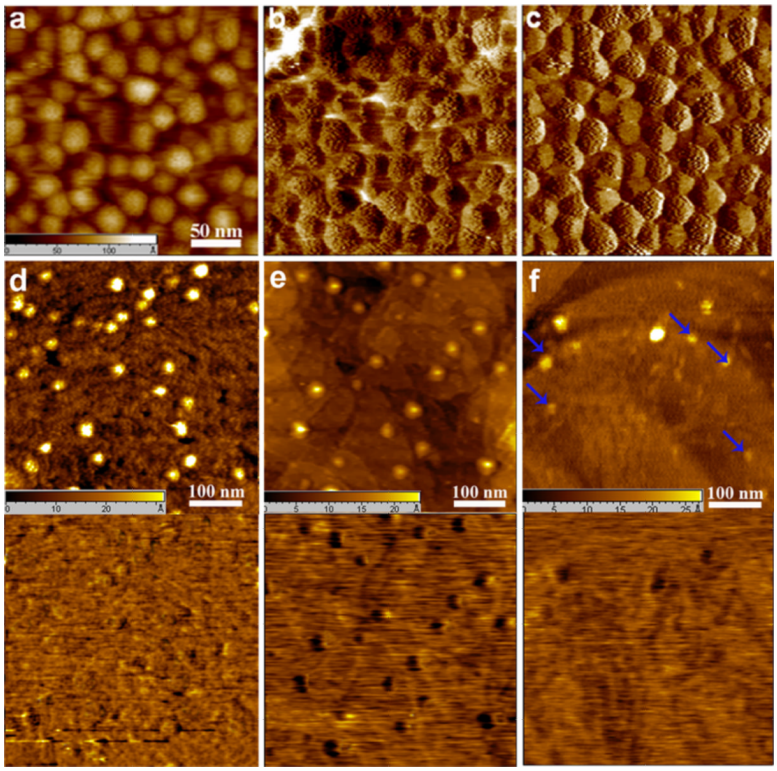


Figure 2.

Ultrahigh resolution and recognition images. The a) topographical, b) amplitude, and c) phase images of micelles **A** using Magnetic AC mode (MAC mode) AFM imaging. Diameters of micelles **A** range from 20–50 nm. d) Topographic (top) and recognition (bottom) images of micelles **A** examined by an unmodified tip showed no recognition events. e) Topographic (top) and recognition (bottom) images of micelles **A** scanned by a 4-dibenzocyclooctynol-modified tip showing recognition events (dark spots) for all micelles. f) Topographic (top) and recognition (bottom) images of a mixture of micelles **A** and **D**, which contain azido or do not have azido moieties, respectively, using a 4-dibenzocyclooctynol-modified tip, showing recognition events only in some of micelles. Micelles that do not exhibit recognition events are indicated by blue arrows in the corresponding topographic image.

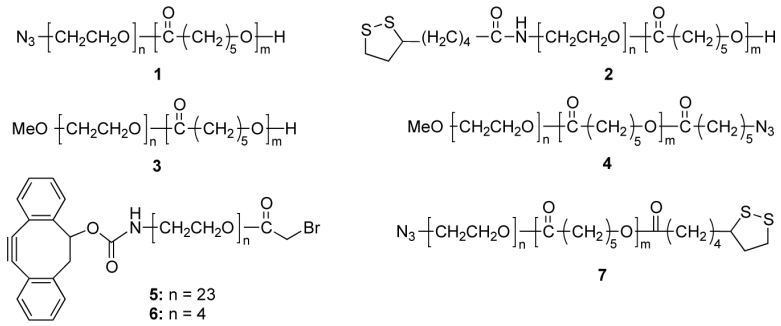


Figure 3.
Chemical structures of the copolymers and click reagents.

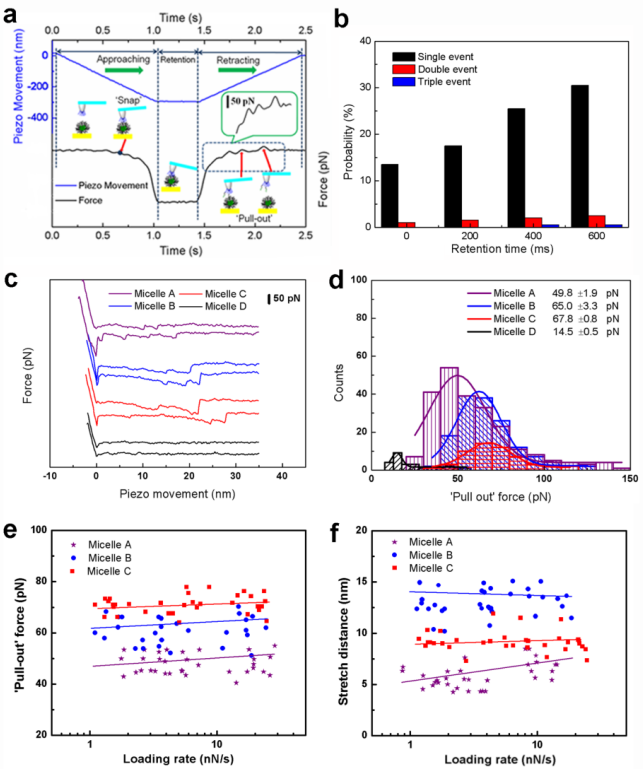


Figure 4.

Force spectroscopy on individual micelles. a) Approach used to establish the relationship between the contact time and number of polymers removed. The movement of the AFM tip can be divided into approaching, retention, and retracting. b) Probability of removal of one, two or three polymers from the micellar surface. c) Representative force-distance curves measured at apparent loading rate of around 15 nN/s using micelle **A**, **B**, **C**, and **D**. d) Histograms used to determine the most probably force to remove a “clicked polymer” from the macromolecular assembly for micelles **A**, **B**, and **C**. e) Typical distribution of the “pulling-out” force depending on the apparent (force) loading rate for micelles **C** (blue, square), micelles **B** (black, circle) and micelle **A** (red, pentagon), the line indicates the most possible magnitude at different force loading rate. f) The typical distribution of stretch distance depending on apparent loading rate for micelles **C** (blue, square), micelles **B** (black, circle) and micelles **A** (red, pentagon), the line indicates the most possible magnitude at different loading rate.

Table 1

Copolymer composition of organo-micelles.[a]

Micelle	Polymer 1	Polymer 2	Polymer 3	Polymer 4	Loading
A	10 %	10 %	80 %		
B	10 %	10 %	80 %		doxorubicin 3 %
C	10 %	10 %	80 %		rhodamine B 9 %
D		10 %	90 %		
E		10 %	80 %	10 %	
F	10 %		90 %		

[a]The composition of the micelles and the loading is expressed as weight percent