

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.

Keywords: atomic force microscopy · azides · imaging agents · mechanical properties · tweezers

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

Herein, 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 electrostatically driven AFM tip is modified with a reagent that can react with a functional group present at the terminus of a macromolecule and form azide-containing polymers. The reaction between the two functional groups 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,[9] 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.

Table 1. Copolymer composition [%] of organomicelles. [a]

<table>
<thead>
<tr>
<th>Micelle</th>
<th>Polymer 1</th>
<th>Polymer 2</th>
<th>Polymer 3</th>
<th>Polymer 4</th>
<th>Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td></td>
<td>doxorubicin: 3%</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td>10</td>
<td>rhodamine B: 9%</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td></td>
<td></td>
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<tr>
<td>D</td>
<td>10</td>
<td>10</td>
<td>90</td>
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<td>E</td>
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<tr>
<td>F</td>
<td>10</td>
<td>90</td>
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</tbody>
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[a] The composition of the micelles and the loading is expressed as weight percent.

Scheme 1. Chemical synthesis of compounds 1-7, reagents and conditions: a) TsCl, pyridine then NaN₃, DMF, 80°C. b) e-Caprolactone, SnOct, 130°C. c) Pd(C), H₂, EtOH. d) d-/l-Thiocetic acid-NHS, TEA, CH₂Cl₂. e) d-/l-Thiocetic acid, DCC, DMAP, CH₂Cl₂. f) N₃(CH₂)₅CO₂H, DCC, DMAP, CH₂Cl₂. g) Et₃N, CH₂Cl₂. h) Bromoacetyl bromide, Et₃N, CH₂Cl₂.

Results and Discussion

Preparation of compounds and micelles: 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. [10] 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(e-caprolactone), 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 dibenzycyclooctynol 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 ca. 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 ca. 45) with caprolactone in the presence of tin(II) 2-ethylhexanoate (SnOct) at 130°C for 24 h gave 1 and 3, respectively \[^{100}\] (m w 26, \(M_n\) ca. 5000, PDI ca. 4). Thiocetic 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-hydroxysucciniminate ester of thiocetic acid in the presence of triethylamine. Compound 4 was obtained by acylation of the hydroxyl of the PCL terminus of 3 with 6-azido-hexanoic 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 ca. 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 thioic acid moiety at the surface, however, the azido groups will be not express 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.

**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 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 (Section SI-2 in the Supporting Information). 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 (Scheme 2). Due to the relatively long polyethylene glycol linker (ca. 9 nm) of 5, it was expected that 4-dibenzyclooctynols attached to the top and cone of the tip would be available for reaction and it was estimated that approximately 1000 molecules would be available for reaction (Section SI-4). High-density modification was achieved by exposing tips to pure (3-mercaptopyrrol)-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 (Section 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

![Figure 2. Ultrahigh resolution and recognition images.](image-url)
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 reaction 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 azide moieties or have azide 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-lapse recognition imaging of micelles A was performed using a tip functionalized with 5 (ca. 9 nm spacer) and 6 (ca. 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 3a and b). Furthermore, the probability of observing force curves resembling the removal of two or three polymers increases 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 (Section 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 nNs⁻¹ (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 with A (5.6 ± 0.2) nm (Figure SI-8-1). The critical micelle concentration for A was higher than that for B and C (Section 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 (Scheme 2) on an Au(1,1,1) surface was formed and examined by AFM imaging using a dibenzylcyclooctynol modified tip. Compound 7 contains a thiotic 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 nNs⁻¹ was measured (from a 1000 distance curves, see Section SI-6), which is about 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.[8]

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 (see 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 catalyst.[10] In another ap-
approach, an AFM tip was modified by a Cu catalyst and employed for spatially controlled reactions of alkynes with immobilized acids.\[8b\]

Here, we demonstrate that distributions, surface topology 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 than 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 behavior of individual molecules, fundamental properties of complex synthetic and biological self-assembled materials can be uncovered.

**Experimental Section**

**Synthesis of copolymer N-PEG-b-PCL (1):** N-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 N3-PEG-OH (9; see Section 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, ca. 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 1H NMR (Figure SI-1–3) relative to the degree of polymerization of the PEO. 1H NMR (CDCl3, 300 MHz) δ = 4.10–4.02 (52H, m; CH₂CH₂CH₂N3), 2.26–2.20 (52H, m; CH₂), 1.65–1.55 (104H, m; CH₂), 1.30–1.22 ppm (52H, m; CH₂). Mn (SEC): 6070 (polydispersity index (PDI) = 1.46). FT-IR spectrum of 1 is shown in Figures SI-1–4.

**Synthesis of copolymer TA-PEG-b-PCL (2):** A suspension of copolymer 1 (500 mg) and 10% Pd/C (100 mg) was stirred under filtration and then dried in vacuum at room temperature to give the product as a white solid (5.5 g, ca. 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 1H NMR (Figure SI-1–3) relative to the degree of polymerization of the PEO. 1H NMR (CDCl3, 300 MHz) δ = 4.10–4.02 (52H, m; CH₂CH₂CH₂N3), 2.26–2.20 (52H, m; CH₂), 1.65–1.55 (104H, m; CH₂), 1.30–1.22 ppm (52H, m; CH₂). Mn (SEC): 6070 (polydispersity index (PDI) = 1.46). FT-IR spectrum of 1 is shown in Figures SI-1–4.
an atmosphere of H2; 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 CH2Cl2 (20 mL) was placed under an atmosphere of Ar and kept cooled (4°C). The solution of TA-NHS (0.3 g, 1.0 mmol; see Section SI-1 for preparation) in CH2Cl2 (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 (CH2Cl2/CH3OH = 1:1 v/v). Concentration of the appropriate fractions (98%) from the chromatography procedure afforded 11 (18.7 mg, 10% yield). 1H NMR (CDCl3, 300 MHz) δ = 4.01–4.02 (2H, m; CH2C=O), 3.85–3.88 (180H, m; CH2OH), 2.38–2.41 (1H, m; S–CH2–CH2–CH–S), 2.26–2.20 (52H, m; CH2C=O), 1.83 (2H, m; S–CH2–CH2–S–CH2–S–CH2), 1.65–1.55 (104H, m; CH2), 1.46 (4H, m; CH2–CH2–CH2–CH2–CO), 1.30–1.22 ppm (52H, m; CH2).

FT-IR spectrum of 11 was shown in Figures S1-1–5.

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 PEG-b-PCL. Briefly, e-caprolactone monomer (3.5 mL, 31.6 mmol) and MeO-PEG-OH (II) (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 a bath filled with liquid-nitrogen and then evacuated, sealed off and kept at 13°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, ca. 90%). The degree of polymerization of the PCL and the polydispersity of the polymers were determined by SEC (Figures SI-1–6). The degree of polymerization of the PCL was also measured by 1H NMR relative to the degree of polymerization of the PEOb. 1H NMR (CDCl3, 300 MHz) δ = 4.0–4.02 (52H, m; CH2C=O), 3.80–3.83 (180H, m; CH2O), 3.38 (3H, s; CH2OH), 2.26–2.20 (52H, m; CH2C=O), 1.65–1.55 (104H, m; CH2), 1.30–1.22 ppm (52H, m; CH2).

FT-IR spectrum of 3 is shown in Figures S1-7.

Synthesis of copolymer PEG-b-PCL[N3]: A solution of polymer 3 (0.5 g, 0.1 mmol) and 6-azido-hexafluorocyclohexane diazonium salt (0.157 g, 1.0 mmol) in CH2Cl2 (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 at room temperature to give the product as a white solid (432 mg, 84%). 1H NMR (CDCl3, 300 MHz) δ = 4.0–4.02 (52H, m; CH2C=O), 3.80–3.83 (180H, m; CH2O), 2.26–2.20 (52H, m; CH2C=O), 1.65–1.55 (104H, m; CH2), 1.30–1.22 ppm (52H, m; CH2).

FT-IR spectrum of 3 is shown in Figures S1-7.

Synthesis of 5: A solution of 15 (100 mg, 0.02 mmol, see Section SI-1 for synthesis) in CH2Cl2 (15 mL) was placed under an atmosphere of argon and then bromoacetyl bromide (40 mg, 0.2 mmol) and NEt3 (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 (CH2Cl2/CH3OH, 30/1, v/v) to afford 5 (66 mg, 67%). 1H NMR (CDCl3, 300 MHz) δ = 7.33–4.96 (8 H, m; aromatics), 5.42 (1H, m; CHOC–O), 4.23 (2H, m; CHBr), 3.67 (2H, m; CH2OH), 3.65–3.57 (180H, m; CH2OH), 3.33 (2H, m; CH2NH), 3.07 (1H, m; ArCH2), 2.89 ppm (1H, m; ArCH2); 13C NMR (75 MHz, CDCl3), δ = 166.5, 154.7, 152.4, 152.1, 129.7, 129.2, 128.0, 127.2, 126.9, 126.5, 126.0, 125.1, 124.9, 122.8, 122.7, 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, 66.6, 65.1, 39.8, 28.6 ppm.

General procedure for the preparation of organomicelles A, D, E, and F: A mixture of 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, 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 4 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 4 h.

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 intensity of 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 nanoparticles 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 µm2. Silicon cantilever tips with a nominal spring constant of about 0.1 N m⁻¹ 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 various different pulling rates, range from 300–4000 nms⁻¹. For each pulling trajectory (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 an Agilent Top Magnetic AC (TOMAC) module in the FM and TFM (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 immobilization methods 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 S1-3a.

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. The organosilicon coated tips were washed with hexane for 15 min using sonication and then immersed in the solution of compound 5 (0.8 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.
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